Editorial introductions

Current Opinion in Rheumatology was launched in 1989. It is one of a successful series of review journals whose unique format is designed to provide a systematic and critical assessment of the literature as presented in the many primary journals. The field of Rheumatology is divided into 15 sections that are reviewed once a year. Each section is assigned a Section Editor, a leading authority in the area, who identifies the most important topics at that time. Here we are pleased to introduce the Journal's Section Editors for this issue.

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Dr Saag is the founding Director of the Agency for Health Care Research and Quality Deep South Center for Education and Research on Therapeutics, the UAB Center of Research Translation in Gout and Hyperuricemia, and the UAB Outcomes Research Center. He also serves as a Vice Chair for the UAB Department of Medicine. He has published over 300 peer reviewed manuscripts; has also authored more than 100 reviews, editorials, and book chapters. Recently he published the first edition of the clinical handbook *Diagnosis and Management of Osteoporosis*.

Dr Saag is the Secretary of the American College of Rheumatology (ACR) and Past President of the Board of Trustees of the National Osteoporosis Foundation.

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Dr Tsokos holds a MERIT Award from the National Institutes of Health and has received several prestigious awards including the Kirkland, Howley, Evelyn Hess awards and the Distinguished Basic Investigator Award from the American College of Rheumatology, the Lupus Insight Award, the Carol Nachman International Prize in Rheumatology and the Marian Ropes Physician Achievement Award. Dr Tsokos' laboratory has opened and led the field of molecular abnormalities on immune cells in patients with SLE and identified previously unknown pathways which have served as the basis for novel treatments which are currently in various phases of development.

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Global research collaboration in a pandemic-challenges and opportunities: the COVID-19 Global Rheumatology Alliance

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Purpose of review
This review discusses the coronavirus disease-2019 (COVID-19) Global Rheumatology Alliance (GRA), the reason for its formation, the challenges with running the registry, and future opportunities for global collaborative research in rheumatology.

Recent findings
The GRA has been successful in collecting and publishing a large volume of case data on patients with rheumatic disease with COVID-19. In addition, the GRA has published reviews, opinion pieces, and patient-directed summaries of research to further assist in disseminating timely and accurate information about COVID-19 in rheumatic diseases. There have been numerous challenges in the journey but they have been addressed through a collaborative problem-solving approach.

Summary
The initial objectives of the GRA to describe the outcomes in patients with rheumatic disease who developed COVID-19 have been achieved. There has been extensive use of the data in the clinic and also to try and understand the mechanisms of disease and opportunities for drug repurposing. There remain numerous important areas for research which the GRA will continue to pursue as the pandemic evolves.

Keywords
coronavirus, COVID-19, observational study, outcomes research, registry

INTRODUCTION

The global coronavirus pandemic presented a huge challenge to the rheumatology community and patients with rheumatic disease. However, it also provided an impetus to create a wide-ranging global research collaboration to address urgent issues [1]. The COVID-19 Global Rheumatology Alliance (GRA) was formed in early March following a conversation on Twitter and had an ethics exempted RedCap registry open for submission 10 days after the project started [2,3]. The mission of the GRA is to collect, analyze, and disseminate information about coronavirus disease-2019 (COVID-19) and rheumatology to patients, physicians, and other relevant groups to improve the care of patients with rheumatic disease. The vision is to bring together the global rheumatology community to curate and disseminate accurate and comprehensive knowledge to advance rheumatology care during the COVID-19 pandemic.

ACHIEVEMENTS

To date the GRA has published a descriptive piece on the first 110 contributed cases [4▪] as well as a study examining the risk factors for hospitalization in a series of 600 patients with rheumatic disease [5▪▪,6]. The most recent work is an analysis of almost 4000 patients examining risk factors for COVID-19 death [7▪▪]. Early data on the lack of protective effect of hydroxychloroquine in COVID-19 was also produced to dispel misinformation about the drug [8▪]. Moreover, a study of the disproportionate impact of COVID-19 on racial/ethnic minorities

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Curr Opin Rheumatol 2021, 33:111–116
DOI:10.1097/BOR.0000000000000783

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KEY POINTS

- The COVID-19 Global Rheumatology Alliance was able to rapidly institute an online case registry to quickly collect case data on rheumatic patients with COVID-19.
- Although there are limitations in the study design the GRA has been able to develop a good picture of how patients with rheumatic disease fare with COVID-19 and direct further work.
- There remain many unaddressed important questions for the field of rheumatology during this pandemic and it is likely the GRA will play a role in helping to address these questions.

who have rheumatic diseases has been published [9▪▪]. These outputs and their key findings are summarized in Table 1. In addition to these published reports from the physician registry, there have been teams of clinicians and scientists working on important questions, who have produced a systematic review and meta-analysis of the use of antirheumatic therapies for the treatment of COVID-19 and a scoping review on the acute respiratory virus adverse events from antirheumatic therapies [10,11]. A review on the treatment of hyperinflammation in COVID-19 was also published [12].

In addition to these important data articles and literature reviews, members of the GRA also advocated for a scientific approach to the assessment of potential COVID-19 therapies to both promote safe clinical practice in the treatment of COVID-19 and also to ensure that medications like hydroxychloroquine were available for patients with rheumatic disease who needed them [13–16]. The survey for patients of their pandemic experiences was also disseminated and results are being analyzed for publication [17].

Wider contributions were also made by those contributing to the GRA with participants contributing to management guidelines including the European League Against Rheumatism (EULAR), Asia-Pacific League of Associations for Rheumatology, and US National Institutes of Health efforts, generating lay summaries for the public, disseminating information to the press and through social media, and completing other studies and reviews relevant to COVID-19 in the rheumatic diseases [18–23].

CHALLENGES

Although the GRA has accomplished a remarkable amount, establishing a global collaboration of this scale over a short period has not been without its unique set of challenges. Here, we discuss some of the challenges encountered and reflect on the processes the collaborative instituted to solve them.

Early challenges

Much of the first several weeks of establishing the GRA involved developing collaborations and infrastructure at record speed.

A key initial challenge was establishing the Institutional Review Board's (IRB) approvals for the

Table 1. Articles published by the COVID-19 Global Rheumatology Alliance (GRA), using data from the physician reported registry

Article	No. of patients	Outcome	Key findings
Gianfrancesco *et al.* [4▪]	110	Descriptive	5% death rate, 35% hospitalization rate
Konig *et al.* [8▪]	80	Descriptive	Hydroxychloroquine use does not prevent COVID-19, or reduce COVID-19 severity
Gianfrancesco *et al.* [5▪▪,6]	600	Hospitalization	Age and moderate/high doses of glucocorticoids increased odds of hospitalization. No increased rate of hospitalization with DMARDs, biologics, or JAK inhibitors at group level
Gianfrancesco *et al.* [9▪▪]	1324	Hospitalization, ventilatory support, and death	Racial/ethnic minorities with rheumatic disease and COVID-19 had increased odds of hospitalization and ventilatory support
Strangfeld *et al.* [7▪▪]	3729	Death	Moderate/high disease activity, rituximab, sulfasalazine, azathioprine, cyclophosphamide, cyclosporine, mycophenolate, and tacrolimus associated with increased odds of COVID-19 death

DMARDs, disease modifying anti-rheumatic drugs; JAK, Janus kinase.

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registry. It was important to develop a flexible data collection system that was easily accessible, secure, and did not require patient consent. During the pandemic, many institutions, including the University of California, San Francisco, committed to expediting IRB review for COVID-19 related projects. This was critical in allowing the GRA IRB to undergo timely review in less than 48 h. Once the initial IRB approval was obtained, a large network of collaborators around the United States and globally worked to adapt the IRB materials for their individual institutions. For example, investigators working in the US Veterans Affairs health systems sought individual institutional approvals and eventually applied for central approval. Globally, we learned that IRB procedures differ substantially from country to country. Some countries, such as Canada, required that individual institutions have separate IRB approvals before participating in the registry. Others, such as the Philippines or Argentina, could obtain central approvals that would apply to all institutions. It was also recognized that strategically and due to European Union General Data Protection Regulations, which have specific data protection, storage, and privacy requirements, a separate provider survey was required for Europe, and a partnership with EULAR was established. The final RedCap survey was provided to EULAR so an identical European registry could be established. The EULAR registry is stored at the University of Manchester (data processor), with EULAR being the data controller. Data from the two parallel registries is combined for analysis.

From the outset, the GRA sought to foster global collaboration. A key challenge was to develop networks where none had existed before. Overcoming this challenge involved using social media in a way that it had rarely been used in rheumatology [24]. Rheumatologists with large followings on platforms such as Twitter quickly disseminated information about the GRA and invited collaborators to communicate on the team platform called Slack. A website was also developed (www.rheum-covid.org) that allowed people to both contribute cases to the registry and access clinical outcome data that had been collected. It also enabled access to proforma documents for IRB approvals and other logistical tasks. This allowed rapid crowdsourcing of work, including the IRB approvals mentioned above and facilitated the recruitment of a series of regional leads around the world. This digital infrastructure was central to the rapid growth and broad participation in the GRA. A similar endeavor was undertaken by EULAR, who developed their own website (https://www.eular.org/eular_covid19_database.cfm).

One important early difficulty was creating a case report form that requested enough information to adequately assess COVID-19 outcomes and clinical and demographic factors, without overburdening busy clinicians. After several iterations, we arrived at a balanced form that could be completed relatively quickly (10–15 min) while providing enough information to allow us to answer the most pressing questions.

An additional early challenge was ensuring scientific rigor and validation despite the compressed project timelines. There were many initial concerns about the integrity of the data. Would rheumatologists complete the case report forms accurately? Would there be duplicate entries? Would someone try to hack the open web-based database platform? Key to overcoming these challenges was having experienced data teams in both Europe and the United States monitoring registry implementation, performing regular data validity checks, developing algorithms to remove duplicates, and instituting procedures to re-contact physicians when data were missing. National investments in scientific infrastructure were critical to this rapid mobilization. In the United States, work relied on the infrastructure already available through a National Institute of Arthritis, Musculoskeletal and Skin Diseases Clinical Research Core, while in Europe, research infrastructure supported by EULAR to support an epidemiology unit was key.

One key challenge was that many of those contributing to the GRA were practicing clinicians. Therefore, at a time when their contributions were often needed clinically, there were also the demands of contributing to the functioning of the GRA. These challenges were addressed with open and honest conversations about what people could and couldn't contribute at any given time. In addition, it was quickly realized that the strengths of the assembled group largely were in clinical and epidemiological research. This led to discussions with the American College of Rheumatology (ACR) and EULAR about both what funding might be available and how that might be best managed. The ACR and EULAR were both able to provide much appreciated logistical and administrative support to enable the members of the GRA to concentrate on the data collection and analysis efforts.

One aspect of the case collection that enabled rapid increases in case numbers was the integration of country-specific registry data. Cases from the French, German, Italian, Portuguese and Swedish registries were transferred and merged with data from the EULAR database, and registries from countries such as Brazil were merged, reducing duplication of effort. The umbrella of EULAR, a truly pan-European organization fostering a multitude of activities in areas of research, patient care, and

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education, facilitated the interaction with national scientific societies of rheumatology (themselves members of EULAR), whereas legal and administrative support from EULAR facilitated practical aspects such as setting up data-sharing agreements with relevant institutions and/or societies. Similarly, research groups globally mobilized quickly to set up data use and transfer agreements to share data with the GRA. The technical aspects of data transfers can be challenging as the various registries use different data collection platforms and formats. This task required interaction of technical teams, mapping of the databases, harmonization efforts, and the creation of export/import tools.

Later challenges

The limitations of a voluntary physician contributed registry are clear, but in the emerging environment of the early days of the pandemic it was very fit for purpose. As the pandemic further evolves it is clear that other study designs are required [25]. The initial data collected by the GRA was valuable in helping define risk profiles and provide comparative data on rheumatic disease patients; however, there remain significant limitations in the way the project is structured. These limitations include the convenience sampling aspect of the physician registry and the lack of comparator groups, both COVID-19 patients without rheumatic disease and/or understanding the denominator for the cases that have been reported. These issues can potentially be addressed by utilizing large health systems and systematically assessing all rheumatic disease patients for infection. This would enable comparisons to be made with both rheumatic disease patients who did not develop COVID-19 and also other patients in the health system who developed COVID-19 but do not have rheumatic disease. The nature of these types of projects is very resource intensive. Groups outside of the GRA are doing some of this type of work already. Moreover, the registry does not capture the patient journey, namely long-term COVID-19 outcomes, such as rehabilitation and recovery data, limiting the ability to view and assess longitudinal outcomes beyond hospitalization, ventilatory support, and mortality.

There is a clear need to improve representation of countries in Latin America, Africa, and Asia in the registry. This will enable region-specific trends to be observed. Often in low-resource settings the medications used and alternatives are very different to those commonly used in Europe and North America so there remains a need to have good knowledge about the patterns of disease and outcomes across all continents and resource settings. The GRA instituted a grants scheme, administered through the International League of Associations for Rheumatology, to support investigators in countries that were underrepresented in the registry. The purpose of the grants scheme was to provide some support to enable the systematic collection of patients with rheumatic disease who developed COVID-19 to enable them to be entered into the database. The grant scheme can also help initiate and support regionally relevant COVID-19 clinical research in areas that lacked the resources to do this previously.

With the constantly growing database of reported cases, there is another growing problem. That of data management, there is a need for a data analytic infrastructure that can assist multiple investigators with different projects. To address region-specific issues, it is important to be able to deliver country-specific data to nations that are using the GRA to perform national studies. The two data hubs that make up the GRA at the University of California San Francisco and the University of Manchester both have excellent infrastructure and have been able to accommodate the growing requests for specific data analytic projects. The GRA has developed transparent, peer-reviewed policies to assist in assigning data analytic resources to the multiple investigators running studies with increasing amounts of information. Ensuring GRA data remains a global and heavily utilized resource needs careful management to ensure the integrity of the data is maintained and high-quality research can continue to be published.

The funding of the alliance is an issue which will likely shape the capacity to branch out into new projects or build on existing projects. Although the initial enthusiasm from industry to support this case was very encouraging and much appreciated, it is almost inevitable that the perceived importance of the alliance will track with the trajectory of the pandemic over time. To branch out into nonpandemic projects, alternate funding sources will need to be found, and the structure and function of the organization will likely have to change to fit both the new projects and new funding sources going forward.

OPPORTUNITIES

During the pandemic

There remain many unanswered questions for rheumatology and patients with rheumatic disease in this pandemic. The emerging questions encompass important areas like vaccines and vaccinations and post-COVID-19 syndromes. These are both important areas for future research effort but it remains to be seen if the GRA, as it is currently configured, is in

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the best situation to lead efforts in these spaces. Both of these research areas will likely benefit from consented research studies with repeated clinical contacts and serial collection of data as well as biospecimens. Therefore it is likely that other study designs are going to be better suited to addressing these important areas. But with the structures and wide network currently in place and a large team with in-depth experience of the rheumatic diseases in the pandemic the GRA is well suited to support efforts in these spaces.

There is also the opportunity to combine data with different registries that are capturing COVID-19 data, particularly those with affinities to rheumatology given mechanistic links in disease pathogenesis or the use of similar drugs (e.g., inflammatory bowel disease: https://covidibd.org/, psoriasis: https://psoprotect.org/). Differences in format and data elements collected may make it challenging to combine data from multiple registries, but collaborating across projects represents an important opportunity to address specific research questions (e.g., specific treatments).

After the pandemic

The opportunity to leverage the existing collaboration that now exists for further topics is exciting. There is likely to come a time when either the majority of the clinical problems presented by the COVID-19 pandemic will have an evidence base to guide them or the impact of the pandemic will be substantially reduced through the uptake of an effective vaccine(s). It will be interesting to see if there is interest in building on the success of the alliance in tackling research related to COVID-19 and turn our attention to tackling other global issues that confront our speciality. We see the strength of the alliance as being able to leverage a global group of clinicians to provide cases with wide geographical distribution. The advantages of this are that low-frequency events may be able to be collected and collated in a way that has never been done before. For example, cases of rare diseases, or rare manifestations of diseases, or low-frequency drug side-effects may be future research directions for the alliance.

CONCLUSION

Although the GRA has achieved much, there is much further work to be done. A major success of the GRA has been the rapid collaborative mobilization of the rheumatology community worldwide. The informal feedback from colleagues that the work of the GRA is being used to help clinicians and patients guide their way through the pandemic is reassuring that we are fulfilling our stated vision. However, there remain many further issues to address in the current pandemic. There might be an opportunity to leverage the existing collaboration to address some of these topics, whereas others can only be addressed by other organizations and more resource-intensive study designs.

Acknowledgements

We wish to thank all rheumatology providers who entered data into the registry and patients who completed the patient survey.

Financial support and sponsorship

The GRA is supported by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR). P.M.M. is supported by the National Institute for Health Research (NIHR) University College London Hospitals (UCLH) Biomedical Research Centre (BRC). J.Y. is supported by NIH/NIAMS P30AR070155.

Conflicts of interest

J.Y. reports personal fees from AstraZeneca, personal fees from Eli Lilly, grants from Pfizer, outside the submitted work. P.R. reports personal fees from Abbvie, Atom Bioscience, Eli Lilly, Janssen, Novartis, Pfizer, Roche, and UCB, and nonfinancial support from BMS, all outside the submitted work. P.M.M. has received consulting and/or speaker's fees from Abbvie, BMS, Celgene, Eli Lilly, Janssen, MSD, Novartis, Orphazyme, Pfizer, Roche, and UCB, all unrelated to this manuscript.
Disclaimer: The views expressed here are those of the authors, and do not necessarily represent the views of the American College of Rheumatology (ACR), the European League Against Rheumatism (EULAR), the (UK) National Health Service (NHS), the National Institute for Health Research (NIHR), or the (UK) Department of Health, or any other organization.

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Racial, ethnic, and healthcare disparities in rheumatoid arthritis

Kevin Yip[a] *and Iris Navarro-Millán*[a,b]

Purpose of review
This review highlights the available data describing racial and ethnic health disparities among patients with rheumatoid arthritis in the United States from an epidemiological, disease activity, and wider socioeconomic standpoint.

Recent findings
Despite centralized government initiatives to include more underrepresentative minority populations into research, many of the studies that examined rheumatoid arthritis still fail to include sizeable cohorts of races or ethnic groups other than whites. Evidence is slowly mounting that individual, provider, and system-level barriers exist and contribute to unequal care that leads to poorer outcomes amongst patients with rheumatoid arthritis. As rheumatoid arthritis is a progressive disease, early treatment is crucial to delay functional decline – a narrow window for many minority patients who are disproportionality affected by disability.

Summary
To combat the inequality that exists amongst rheumatoid arthritis patients we must focus on why discrepancies exist on every level, system, physician, patient, and illness. Further research is needed to tease the complex interplay between race, social economic status, medical access, and outcomes to explain the disparities found in rheumatoid arthritis.

Keywords
access, disability, ethnic, health disparities, healthcare, racial, rheumatoid arthritis, socioeconomic

INTRODUCTION

Health disparity in the United States has multiple dimensions and represents differences in health outcomes between different groups of society [1]. These are not limited to race and ethnicity, but can also be on the basis of sex, sexual identity, disability, or age [1]. Working toward health equity in society requires that every aspect of society is involved to address inequalities between social groups so that they can attain the maximum level of health among all people [2]. Since 2010, health disparities have been addressed as a priority by the US Department of Health and Human Services through the Healthy People plan [1].

Racial, ethnic, and access-related health disparities are not the exception among patients with rheumatoid arthritis; one of the most common inflammatory arthritis in the United States. Nevertheless, disparities related to this disease specifically have not been studied in as much detail as in other conditions (e.g., lupus, diabetes); hence, there is not a clear understanding about the magnitude of this problem or even the best way to intervene to address issues related to social determinants of health. This review summarizes the data available to date regarding racial and ethnic disparities among rheumatoid arthritis patients within the United States.

EPIDEMIOLOGY

The prevalence of rheumatoid arthritis is approximately 1% in the US population [3,4]. For comparison, mean age-adjusted prevalence rates suggest that North Africa, Middle East, and Asia have relatively lower prevalence at 0.16%, North America and Western Europe at 0.44% with Australasia

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Curr Opin Rheumatol 2021, 33:117–121
DOI:10.1097/BOR.0000000000000782

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KEY POINTS

- Rheumatoid arthritis research continues to predominantly focus on white populations leaving many questions such as epidemiology, disease course, and outcomes unanswered in racial minority patients.
- Evidence is mounting that the differences observed between rheumatoid arthritis in racial groups can be attributed to nonbiological factors.
- Socioeconomic status, differences in therapeutic prescriptions, and access to healthcare reveal significant health disparities which must be addressed to provide equitable healthcare for all patients with rheumatoid arthritis.

having the highest at 0.46% [5]. In the United States, specific populations have been identified with higher rates of rheumatoid arthritis including the Pima and Pagago Indians of the American Indian population who have an age-adjusted prevalence of 5.3% [6]. Although it is traditionally reported that rheumatoid arthritis affects predominantly whites, it is important to examine the proportion of individuals with rheumatoid arthritis within each racial/ethnic group. According to the 2019 U.S. Census Bureau, the total U.S. population was 328 239 523 with 76.3% whites, 13.4% African-American, 18.5% Hispanic or Latino, 5.9% Asian, 2.8% listed as two or more races, and 0.2% Native American and other Pacific Islander [7].

To date, the majority of the epidemiologic studies, outcomes, and trials of patients with rheumatoid arthritis included primarily white patients or without racial information defined [3,4,8]. A recent systematic review of 240 rheumatoid arthritis Randomized control trial (RCTs) estimated an overrepresentation of the white population ranging between 74.6% in 2010 to 97.0% in 2013 of researched participants [9▪▪]. There is little population-based data regarding the specific incidence rates and prevalence of rheumatoid arthritis among individuals who are African-American or Hispanic. This lack of understanding of the epidemiology of rheumatoid arthritis by race and ethnicity limits our understanding of the burden of rheumatoid arthritis among different groups of our society and the health disparities amongst patients.

DISABILITY AND DISEASE ACTIVITY

Race itself appears to reveal a discrepancy between arthritis and related disability. Given the correlations between ethnicity, race, social-economic class, and even culturally this can be difficult to interpret [10].

The differences in reported disability could be related to differences in treatment for rheumatoid arthritis that could have led to disability. Still, once individuals with rheumatoid arthritis are disabled, they might still experience an additional disparity in the way that rheumatoid arthritis is treated. In a recent study among dual-eligible (Medicare and Medicaid) beneficiaries of Social Security Disability Insurance (SSDI), individuals who filed for disability benefits before the retirement age of 65, showed significant differences in the use of biological Disease-modifying antirheumatic drug (bDMARDs) amongst races [11▪▪]. African-Americans were least likely to receive bDMARDs (49.3%) than whites (53.3%) whereas Hispanics were more likely to receive bDMARDs (60.9%) [11▪▪]. These differences persisted after controlling for social determinants of health [11▪▪]. Disability in rheumatoid arthritis has led to high opioid prescription with more than 66% of SSDI beneficiaries to receive chronic opioids [11▪▪]. This proportion was not different between African-Americans and whites beneficiaries of the SSDI by 2014. This data suggest that early disability can result in overreliance on opioids, likely given to high level of disability in a group of patients who were to begin with, highly vulnerable [11▪▪].

There are several studies that examined rheumatoid arthritis disease activity by race and ethnicity. Greenberg et al. analyzed data from 6008 patients across community-based rheumatology clinics over a five-year period in a cross-sectional study [12]. Although there was improvement in disease activity across all racial groups, there were only small differences noted in clinical disease activity index scores between whites 12.38 (11.36–13.4), African-Americans 13.75 (12.39–15.1, $P=0.007$), and Hispanic patients 13.01 (11.68–14.34, $P=0.179$) in an adjusted model accounting for practice setting, treatments, and patient sociodemographic factors [12]. In another cross-sectional study focused on one academic center practice, African-Americans patients had increased disease activity score (DAS) scores than whites (5.5 ± 1.3 versus 4.3 ± 1.4; $P<0.001$) and Health Assessment Questionnaire (HAQ) (1.5 ± 0.8 versus 0.9 ± 0.7; $P<0.001$); however, after accounting for socioeconomic, demographic, and behavioral influences, race was not independently associated with the reported differences. Hence, to date, there is little data to support that individuals from a specific racial or ethnic group have more aggressive or higher disease activity in rheumatoid arthritis [13,14].

Jordan *et al.* analyzed 100 female patients with rheumatoid arthritis and found that African-Americans had less physical activity and more negative affect compared with their white counterparts

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despite no difference in pain severity [15]. They found that the two groups used different psychological coping strategies with white patients more likely to ignore pain, whereas African-Americans patients turned to praying and hoping. Regardless of racial background, coping statements better predicted pain control suggesting that racial differences in coping strategies may contribute to reported differences [15]. Given the history and racism that African-Americans and other minorities encountered in the healthcare system and in their communities, these coping mechanisms could have been shaped by their experiences with unequal treatment and racism that white individuals did not experience. This issue constitutes a problem that is not only limited to the healthcare system, but to the political system and how our society addresses inequality within its members.

SES/ACCESS

A cross-sectional study comparing a cohort of 4730 rheumatoid arthritis patients found that white patients, despite having a longer disease course had better global health scores and less pain [16]. Level of education attainment, duration of rheumatoid arthritis diagnosis, and number of other comorbidities were found to impact the pain ratings of Hispanic and African-American patients [16]. There is convincing data across the world that lower social economic status (SES) (measured in different ways such as gross income, occupation, educational level, and area of residence) has been linked to worse disease in rheumatoid arthritis patients, from disease activity, pain, and disability [17▪,18,19]. Growing evidence has suggested that low SES even in childhood has a statistically significant trend ($P < 0.0001$) of increasing the risk of development of rheumatoid arthritis; food insecurity (odds ratio = 1.5), young maternal age (<20 versus 20–34 years; OR = 1.7), and childhood household education (<12 years versus college degree; OR = 1.7; 95%) [20].

Schmajuk *et al.* looked at 93 143 Medicare-enrolled patients with rheumatoid arthritis and found significant correlations of Disease-modifying antirheumatic drugs (DMARD) use with socioeconomic factors [21]. Living in an area of low SES, having low personal income (defined by needing state assistance for their Medicare Part B), male gender and African-Americans race were all associated with a lower likelihood of being prescribed a DMARD [21]. As early treatment is beneficial for earlier remission, prevention of joint damage and disease-related disability [22–24], it stands to reason that minorities without equitable access to care will accrue and be exposed to longer uncontrolled disease before therapy and therefore have poorer prognosis and remission rates.

Kerr *et al.* compared the treatments of 2899 patients in the Veteran Affairs Rheumatoid Arthritis Registry (VARA) in the VA healthcare system (akin to a form of Universal Healthcare in the U.S.) and patients in the Ethnic Minority Rheumatoid Arthritis Consortium (EMRAC) where patients were part of varied healthcare systems [25]. Notably, in the VARA cohort, there was no difference in biologic use between racial groups, whereas EMARAC white patients had a higher odds ratio of 1.66 of receiving biologics compared with their nonwhite counterparts [25].

In a longitudinal observational study of 8545 patients with rheumatoid arthritis, 43.6% noted difficulties paying out of pocket medical expenses. Those who had the greatest difficulty with healthcare costs were more likely to be on social security disability (33.9 versus 10.1%), be a minority race (10.3 versus 5.1%), have worse HAQ Scores (1.5 versus 0.9), higher comorbidity index (2.5 versus 1.6), less likely to be a college graduate (20.3 versus 36.1%) and at the poverty level defined by Health and Human Services poverty guidelines (51.3 versus 12.3%) compared with those who reported 'no difficulty' with medical costs. Patients most at financial risk were also those disproportionately affected those with the most severe disease and twice as likely to be a racial minority [26]. This may go some way to attribution of reduced treatment rates and access to effective therapies in this most vulnerable patient group. In addition, out of pocket costs have been shown to negatively impact adherence to medications in rheumatoid arthritis [27▪] and represent another barrier to those in lower SES which are made disproportionately of minority populations especially as they are almost twice as likely as white patients to be work-disabled [28].

SYSTEM LEVEL DISPARITIES

Barton *et al.* [29] examined a diverse racial and immigrant population served by the same rheumatologists that worked at both a University and public based clinic. This study found that whites, English speaking, and nonimmigrant patients had lower DAS-28 and HAQ at a University setting only [29]. Notably, these differences were still present after adjustment for medication use suggesting that therapies alone did not account for the discrepancies but instead suggests the presence of a health disparity on the basis of clinic-level differences. Clinic-level differences could contribute to the health disparities observed in the patients with rheumatoid arthritis such as variation in time to initial rheumatology care and access to treatment [29].

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Looking at two affiliate rheumatology clinics within one medical school, Suarez-Almazor *et al.* [30] compared their African-American, Hispanic and white rheumatoid arthritis patients and found that non-white patients were more likely to be in a public rather than private clinic (83 versus 18%) and wait significantly longer before being started on DMARD therapy (median of 7 versus 1 year). In patients with relatively early disease (<5 years), more whites than non-white patients had already tried some form of DMARD prior to their index visit (64 versus 32%) [30]. These findings suggest intrinsic biases, which prevent the same level of access and care to racial minorities, are a crucial barrier in rheumatoid arthritis where early treatment can prevent long-term joint damage [22–24].

As medicine moves toward an evidence-based approach, it is important to reflect upon the long-standing problem to recruit representative populations into research studies. The data and results they generate help guide societal guidelines and standards of practice especially as it is often the underserved minorities and often those in lower SES brackets that miss out on only research opportunities and potential future benefit. Notably, most large-scale studies of rheumatoid arthritis have been performed in Western nations, which skews identification of risk factors. Despite making up almost 41% of the US population, racial minorities only represented 16% of the rheumatoid arthritis population for RCTs [9▪▪]. Concerningly, despite efforts to increase awareness and participation from both researchers and patients, there hasn't been an obvious improvement of minority representation over the 10-year period studied (2008–2018) [9▪▪].

Given the differences and burden of disease on non-white populations delineated in this article, it is imperative that we better understand why discrepancies exist on every level, system, physician, patient, and illness level, if we are to fix the problem. This will require us to revisit the policies that contributed to bring us and our healthcare system to this unequal state to begin with, so we can right this wrong.

Acknowledgements

None.

Financial support and sponsorship

I.N.M. work was supported by K23-AR-068449 from the National Institute of Arthritis and Musculoskeletal and Skin Diseases.

Conflicts of interest

I.N.M. has received speaker fees from SOBI pharmaceuticals. K.Y has no conflicts of interest to declare.

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Scleroderma epidemiology update

Leonardo Martin Calderon[a] *and Janet E. Pope*[b]

Purpose of review
Systemic sclerosis (scleroderma, SSc) is a rare multisystem autoimmune disease characterized by autoantibodies, vasculopathy, and fibrosis of the skin and internal organs. This review aims to provide an overview and summary of the recent epidemiological studies in systemic sclerosis.

Recent findings
Global trends of scleroderma demonstrate greater prevalence of SSc in European, North, and South American patients compared with East Asian patients. However, the greatest prevalence (47 in 100 000), was found among the indigenous peoples in Canada. Phenotypical differences exist depending on the age of presentation with greater internal organ involvement and disease acceleration present in older patients. Sex differences include greater severity of disease expression, relative prevalence of diffuse cutaneous SSc, and organ involvement in males versus females. New studies conflict with previous data reporting greater proportion of pulmonary arterial hypertension in females. Furthermore, the effect of low median household income is demonstrated as a factor increasing risk of death in SSc patients.

Summary
Understanding the epidemiological factors in SSc enables patient care through patient classification, prognostication, and monitoring. Future research may emphasize enrichment of SSc patients in randomized trials who are more likely to progress or be treatment responsive, focused screening, and personalized patient care through the creation and validation of new SSc criteria and subsets.

Keywords
autoimmunity, epidemiology, mortality, scleroderma, sex differences, systemic sclerosis, systemic sclerosis

INTRODUCTION

Scleroderma, or systemic sclerosis (SSc), is a rare multisystem autoimmune connective tissue disease characterized by vasculopathy with skin and internal organ fibrosis and autoantibodies [1,2]. SSc is arranged into subsets including limited and diffuse cutaneous SSc (lcSSc and dcSSc) [3]. SSc has high morbidity [4,5], decreased quality of life [6], significant societal economic burden [7,8], and increased mortality [4,5,9]. Traditionally, SSc patients are predominantly female with an increasing age of onset and at greater risk of developing lcSSc, peripheral vascular disease, and pulmonary arterial hypertension (PAH) [10,11]. Conversely, men have greater risk proportionately of developing dcSSc, with worse interstitial lung disease (ILD) and cardiovascular complications. The purpose of this review is to highlight the newest literature relating to the epidemiology of SSc.

DEMOGRAPHICS OF SYSTEMIC SCLEROSIS

The classification criteria used to identify SSc cases in population-based studies may vary. Criteria include the preliminary 1980 American College of Rheumatology (ACR) criteria [12], the 2001 LeRoy and Medsger criteria in early SSc [13], and the 2013 ACR and European League Against Rheumatism (EULAR) criteria [14]. Differences in sensitivity and specificity between these criteria are well documented [15].

Incidence and prevalence

Demographic parameters in SSc vary with gender, ethnicity, and geography. Zhong *et al.* [16▪▪] synthesized incidence and prevalence patterns of in SSc across North America, Asia, Australia, and South

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Curr Opin Rheumatol 2021, 33:122–127
DOI:10.1097/BOR.0000000000000785

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KEY POINTS

- Younger age of SSc delineates a greater risk of developing dcSSc subset. However, disease duration and age increase the risk of death overall.
- SSc is more prevalent in women versus men (approximately 4–1). There is increased organ involvement and greater risk of developing dcSSc in males.
- Adjusted analyses of socioeconomic factors in patients with SSc demonstrated that a lower median household income was associated with an increased risk of mortality.
- The development of new classification criteria using new tools, such as proteomics or epigenomics, may optimize personalization of SSc patient care, patient monitoring, prognostication, and research cohort creation.

America. Original population-based observational studies were included. Prevalence in European populations was greater (10–35 per 100 000), than in East Asian populations (3.8–5.6 per 100 000). Prevalence in South America was 29.6 per 100 000. However, the highest prevalence was observed in the indigenous people of Canada (47 per 100 000). Pooled prevalence of SSc was 23 per 100 000. Furthermore, incidence in East Asian populations was 1.09–1.5 in 100 000, 1.5 per 100 000 in Australia, 2.1 per 100 000 in South America, and in European populations ranged from 0.77 in Netherlands, to 4.3 in Italy per 100 000. This review summarized prevalence and incidence parameters in SSc worldwide.

Age

SSc age of presentation can vary but commonly occurs in middle age with increasing age over time. Importantly, differences in disease severity have been described depending on age of presentation. Carreira *et al.* [17] analyzed patients with early SSc in three age groups through a cross-sectional analysis of the EULAR Scleroderma Trials and Research database (EUSTAR). Patients were identified using the 1980 ACR criteria with less than three years from the first non-Raynaud's phenomenon SSc symptom. They categorized age strata as less than 30, 31–59 years, and at least 60 years old. The study identified 1027 patients of whom 90% were whites with 80% females. Younger patients had higher anti-Scl-70 antibodies (53 versus 35 versus 30%) and higher likelihood of having dcSSc (54 versus 40 versus 34%) compared with the medium and older patient strata. Conversely, older patients were more likely to have lcSSc (58 versus 53 versus 35%) with more cardiac involvement including conduction blocks (15 versus 6 versus 6%) and diastolic dysfunction (26 versus 12 versus 3%) comparing descending age strata, respectively.

Another publication by Moinzadeh *et al.* [18▪] also reported that older patients had more lcSSc in a subgroup analysis of 3281 patients from the German Network of Systemic Scleroderma. One quarter of their cohort developed SSc at age more than 60 years. Within the lcSSc and dcSSc subgroups, they found an increased frequency of organ involvement in the lungs and heart in older patients (pulmonary hypertension in lcSSc and dcSSc, pulmonary fibrosis, and cardiac involvement in the dcSSc subset) and acceleration of disease progression in older patients. Although there were less digital ulcers in the older onset patients, these observations increase our understanding that older onset SSc patients may have a worse disease course.

Jiang *et al.* [19▪] performed a systematic review examining the factors associated with PAH in SSc. Studies included had a sample size larger than 20 comparing SSc patients with PAH identified by right heart catheterization to those without PAH. The risk factors most often cited that were associated with PAH in SSc were older age, lcSSc subset, longer disease duration, positive anticentromere antibodies, and telangiectasia.

These studies demonstrate differences in clinical features and disease progression secondary to age of symptom onset and diagnosis.

Gender differences

A review by Hughes *et al.* [20▪▪] investigated gender differences in SSc. Generally, SSc has a female-to-male ratio between 3:1 and 7:1 with some geographical exceptions demonstrating ratio reversal of male to female from 4.7:1 in North East England to 14.5:1 in Tokyo. Males predominantly present with dcSSc compared with females (61 versus 34%). Females have more lcSSc (57%) compared with males (35%). Time to diagnosis of dcSSc after onset of Raynaud's phenomenon is slightly longer for women than in men (1.1 versus 0.8 years). Men had more SSc associated cardiomyopathy, left ventricular dysfunction, ILD, and scleroderma renal crisis. PAH is prevalent in both sexes; however, postmenopausal women had more isolated PAH (Group 1 PAH). Some gender differences are corroborated by this review including greater severity of disease expression, prevalence of dcSSc, and lung and heart organ involvement in males. However, evidence of greater proportion of PAH in females remains conflicting.

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Hormonal differences

Estrogens, particularly estradiol, have been previously implicated as profibrotic agents in SSc. Ciaffi *et al.* [21▪] performed a systematic review investigating the role of sex hormones in SSc. Studies of SSc by any definition/classification were included as were case reports, case series, cohort studies, and registries. In general, the quality of included studies was poor. Estrogen may have profibrotic effects and in postmenopausal women with SSc, there is less skin involvement. There may be a vasodilatory effect of estrogen and some data suggest that a hypoestrogenic state increases the risk of developing PAH, whereas hormonal replacement therapy in menopause might be protective against PAH. There is likely insufficient data to draw conclusions and confounding where age is a risk factor for PAH.

Furthermore, Frost *et al.* [22▪] enrolled and analyzed the estradiol levels of 83 males aged 50 or older with recent onset dcSSc (within 2 years of first symptoms) and compared them to 37 healthy male controls and postmenopausal age-matched women with dcSSc. The men with dcSSc had higher levels of estradiol compared with healthy males and postmenopausal dcSSc at 30.6, 12.9, and 24.2 pg/ml, respectively. High estrogen levels (compared with low levels) in dcSSc men over the age of 50 were associated with more skin fibrosis progression, increased cardiac involvement, and reduced survival. Other studies are needed to corroborate these findings.

Ethnicity/race

African-American patients have been previously described to be younger at disease onset with a greater likelihood of having dcSSc, and increased mortality compared with non-African-Americans [23,24]. Moore *et al.* [25▪▪] performed a retrospective study comparing African-American versus non-African-American SSc patients matched for sex, age, disease duration, and SSc subset. Median household income derived from residence zip code was used as a surrogate of socioeconomic status (SES). In the unadjusted analysis, African-American ancestry did demonstrate an elevated hazard ratio of 2.1 ($P = 0.006$) for death during follow-up. However, there were findings that differed from previous publications including no difference in age at initial visit for SSc and prevalence of dcSSc. African-American ancestry when adjusted for age, sex, disease duration, SSc subset, and anti-Scl-70 status was not predictive of mortality. SES variables, such as marital status, education, insurance type, employment status, and imputed household income were studied and a lower household income increased mortality. This study demonstrates the impact of socioeconomic status on mortality in SSc. Findings in the literature may differ depending on whether other important disease and SES factors are adjusted for.

Environmental and occupational exposures

In addition to health behaviors, occupational and environmental exposures may account for some differences in SSc severity. In a review by Marie *et al.* [26], environmental and occupational exposures implicated in the modulation of the epigenetic determinants in SSc development and progression were collated. Occupational exposure to crystalline silica and organic solvents, such as aromatic or chlorinated compounds is strongly associated with SSc development. Exposed patients are at higher risk of developing dcSSc, digital ulcers, and interstitial lung disease. Additionally, exposure to heavy metals including antimony, cadmium, lead, and mercury seems to increase SSc incidence. Pathogenic mechanisms of exposure may include reactive oxygen species and endothelial dysfunction. Antimony and platinum in males, although antimony, mercury, lead, cadmium, palladium, and zinc in females were associated with SSc. Occupational differences may affect associations in men and women differently. For instance, men are far more likely to work in mining. Some patients receive compensation if their employment seems to be a strong risk for the development of SSc, so taking a detailed occupational history in people with SSc may be important.

Mortality

The mortality rate is greater in SSc than in the general population with the leading causes of death being ILD and PAH [27,28▪]. Lee *et al.* [29] performed a metaanalysis for standardized mortality ratios (SMR) in SSc patients. Cohort studies with predefined SSc criteria and reporting overall, sex, and/or disease subtype-specific SMRs were included. The SMR in SSc patients was 2.8 (95% confidence interval 2.2–3.6, $P < 0.001$). No significant differences in SMR between men and women with SSc were found (3.5 and 2.9, respectively). SMR was nearly five times higher in the dcSSc subset compared with lcSSc at two times higher than the age and sex-matched general population. This study differs from some previous findings as male sex did not confer an increased SMR above women with SSc overall (numerically higher but not statistically) and in the dcSSc subset.

Prognostic factors affecting disease progression and mortality in dcSSc were studied by Becker *et al.*

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[30▪▪]. Using the EUSTAR database, those early dcSScs who either had a follow-up visit or died within 12 plus-minus 3 months after baseline were included. Disease progression was defined as either new onset renal crisis, decreased forced vital capacity greater than or equal to 10%, new left ventricular ejection fraction (LVEF) less than 45% or decreased in LVEF greater than 10% for patients with baseline of lower than 45%, new onset echocardiography-suspected PAH, or death. They found associations between disease progression and older age, active digital ulcers, lung fibrosis, muscle weakness, and elevated C-reactive protein. This supports the study of older age onset SSc had a worse prognosis [18▪]. Early skin fibrosis progression was associated with decreased lung function and worsened survival in patients with dcSSc using EUSTAR data as reported by Wu *et al.* [31].

LIMITED CUTANEOUS SYSTEMIC SCLEROSIS

Despite comprising two-third of SSc patients, inclusion of lcSSc subset in research is less compared with dcSSc [32]. Although PAH trials often have more lcSSc patients, many trials include only early dcSSc or progressive dcSSc patients and ILD trials comprise more dcSSc patients. Some of this reflects the epidemiologic differences between the subsets and trials to improve skin will include only those with higher skin scores (i.e., the dcSSc subset). Frantz *et al.* [33▪] analyzed observational data from EUSTAR to identify factors predictive of progression of skin and lung fibrosis and vasculopathy in patients with lcSSc who met the 2013 ACR/EULAR criteria. A total of 8013 lcSSc and 4786 dcSSc patients were analyzed. Skin progression was minimal in lcSSc versus dcSSc, at 3.8 and 14.9%, respectively. Nearly half of the lcSSc patients who had skin progression developed proximal or truncal disease and were reclassified as dcSSc. Lung fibrosis increased in lcSSc patients over three years of follow-up and changes in forced vital capacity in lcSSc patients mirrored dcSSc changes. This study illustrates the necessity for pulmonary monitoring in both subsets of SSc.

CLASSIFICATION/DIAGNOSIS

Systemic sclerosis subtype criteria

The classification and subsetting of SSc by LeRoy *et al.* [34] has been useful in risk stratification, treatment guidance, prognosis ascertainment, and grouping of patients for research purposes [35]. However, updated classifications guided by new tools, such as metabolomics, proteomics, genomics, transcriptomics, and epigenomics, may optimize personalization of SSc patient care. For example, Smeets *et al.* [36] derived three distinct pathophysiological 'fingerprints' through antibody clustering with associated vascular and inflammatory mediators. Moreover, Johnson *et al.* [37▪] reported on the evolution and ongoing development of new SSc classifications. It was recommended that new classification systems should meet three principal requirements: enriched research cohorts and communication among practitioners; improved clinical care with specific investigations, monitoring, and therapies; improved understanding of prognosis with special attention to development of internal organ involvement and survival. An example of a new classification system is presented in Nihtyanova *et al.* [38▪] with seven subtypes, clustering patients by skin extent and antibody profiles to predict morbidity and mortality. Nevertheless, new classification schemes will need to be proven superior, to previous subsets and reliable, feasible, and valid prior to implementation.

Undifferentiated connective tissue disease at risk for systemic sclerosis

Valentini and Pope [39] described associated features of patients with Raynaud's phenomenon at risk of developing SSc. Antibodies are quite predictive of developing SSc in patients with Raynaud's phenomenon including positive Scl70, anti-nuclear antibody at least 1/320, anticentromere antibody and avascular areas on nailfold capillaroscopy. There is nonstandardized terminology for undifferentiated connective tissue disease at risk for SSc including 'prescleroderma' and other terms.

Antibody profiles and human leukocyte antigen associations in systemic sclerosis

Phenotypical differences in SSc have been proposed to be partially related to genetics and antibody profiles. Gourh *et al.* [40▪] investigated human leukocyte antigen (HLA) and antibody profile subsets in African and European-Americans. HLA-DRB1∗ 08:04 and HLA-DRB1∗ 11:02 were identified as being of predominantly African ancestry, whereas HLA-DRB1∗ 08:04 was associated with the poor-prognosis antifibrillarin antibody subset of SSc and increased the risk of SSc seven times [41]. Additionally, HLA-DPB1∗ 13:01 was associated with antitopoisomerase antibody in a third of SSc patients regardless of ancestry suggesting a pathogenetic role beyond ancestry. Therefore, this study illustrates the role of HLA alleles and their associated antibody profiles and contributes to the explanation of why some

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studies have observed more severe disease in African-American patients; however, another study did not show worse outcomes in this population when adjusting for SES factors [25▪▪].

CONCLUSION

SSc is a rare multisystemic autoimmune disease with vasculopathy and fibrosis. This review helps to refine epidemiological patterns within SSc. SSc has a pooled prevalence of 22 cases in 100 000 from many studies with variable rates depending on country and race/ethnicity. Older age onset in SSc is associated with accelerated disease progression and internal organ involvement. Novel sex differences in SSc include ratio reversal from the classic female predominance in some specific patient populations and different distributions of lcSSc and dcSSc subsets and estradiol levels may impact prognosis in men. Lower household income in SSc is associated with worse outcomes in the USA. Ultimately, new classification subsets, guided by new diagnostic tools and improved epidemiological and biological SSc disease understanding, will facilitate superior and personalized care and research within SSc.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

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- ▪ of special interest
- ▪▪ of outstanding interest

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Adherence to gout guidelines: where do we stand?

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Purpose of review
Although gout is a common, well-recognized, and extensively researched rheumatologic disease, it continues to be underappreciated and undertreated. Although the prevalence of gout has been rising over the past several decades, adherence to urate lowering therapy continues to be suboptimal. Recent studies have underscored the potential success of guideline-directed therapy.

Recent findings
Adherence to gout treatment continues to be suboptimal according to multinational metaanalyses. Moreover, studies measuring adherence are prone to overestimation and each methodologic approach has intrinsic limitations. Adherence may be analyzed from the perspective of patient adherence to taking a medication, or provider adherence to treatment guidelines. In addition to considering traditional risk factors, adherence should be viewed through the lens of healthcare disparities. The RAmP-Up trial and Nottingham Gout Treatment trial demonstrate the success of protocolized gout treatment using existing guidelines for reference.

Summary
Standardized gout treatment protocols should be established for all primary care and specialty practices. Two successful methods of improving adherence include using nonphysician providers to coordinate urate lowering therapy titration and monitoring serum urate. Having more frequent outpatient visits to focus on direct patient care and education has also been successful.

Keywords
compliance, gout, guideline, treatment

INTRODUCTION: SCOPE OF THE PROBLEM

Gout is the most common inflammatory arthritis in the United States, affecting more than 8 million individuals [1]. Gout prevalence approaches 4% worldwide, and as high as 6% in specific subpopulations [2,3]. Although gout presents commonly as acute flares, many patients develop a chronic polyarticular arthritis characterized by chronic pain and joint deformities.

In the United States, gout leads to about 114 000 disability-adjusted life years lost, about 3.9 million ambulatory care visits and about 175 000 emergency room visits annually [4,5,6], with an average of five days of lost work and $3000 of excess healthcare spending per person per year [5,7]. Considering both direct and indirect impacts, the US annual cost of gout care exceeds $6 billion [5]. Owing to its high prevalence, high morbidity, and high cost, effective treatment of gout according to guidelines is a desirable and achievable goal. Unfortunately, gout treatment remains substandard because of patient nonadherence and poor physician performance. Here, we provide an updated review of gout treatment adherence, and potential improvements that can be made in gout care.

METRICS FOR ADHERENCE TO THERAPY

Studies assessing adherence to urate-lowering therapy (ULT) commonly employ two metrics. Nonpersistence is defined as a gap in therapy, typically more than 30 or more than 90 days. Adherence is classified as a patient taking their ULT at least 80% of the time during a study period.

Medication utilization data are variously obtained through electronic medical records, claims data, electronic monitoring devices/pill counts, and/or self-reporting. Each approach has significant limitations, leading to variable study results. Medication adherence is often measured by a surrogate marker, 'portion of days covered' by prescriptions.

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Curr Opin Rheumatol 2021, 33:128–134
DOI:10.1097/BOR.0000000000000774

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KEY POINTS

- Adherence to urate lowering therapy continues to be poor worldwide.
- Guideline-directed gout care is underutilized in primary care and rheumatology.
- Frequent outpatient visits, patient education, and shared decision making are effective tools in gout management.
- Community, race, ethnic, and economic disparities adversely affect gout treatment adherence.
- Incorporating guideline-driven protocolized care has been the most effective intervention to date.

However, this metric does not account for prescriptions filled but not consumed by the patient. Furthermore, successful prescriptions require provider initiation and refills, such that 'portion of days covered' is effectively a marker of prescriber performance [8]. On the other hand, patient self-reports tend to overreport compliance. Even the use of Medication Event Monitoring Systems (MEMS; medication bottles with caps that record when the bottle is opened) may be biased by the Hawthorne effect (patient awareness of ongoing measurement improving their adherence). All in all, measures tend to underestimate the extent of actual nonadherence.

PATIENT ADHERENCE TO URATE-LOWERING THERAPY

Prescription data, claims-based data, and medical records allow for research on large samples and discovery of overarching trends. Multinational systematic reviews and metaanalyses report ULT adherence rates around 46–47% and nonpersistence rates ranging from 54 to 87% [8,9,10]. However, epidemiologic studies from Israel and the US report ULT adherence rates at 17 and 18%, respectively [11,12]. An Italian claims-based study demonstrated ULT adherence rates of 46% at one month and a significant drop to 3% at one year [13]. Another study from Ireland found an increase in nonpersistence from about 50% at six months to 75% at one year [14]. Studies measuring adherence to ULT indicate declining rates over time, a trend that was expected to continue beyond the study end [13,14]. Table 1 summarizes studies that address adherence to ULT.

Studies using direct measures of medication utilization (e.g., prospective studies) have found strikingly higher adherence when compared with studies analyzing records from databases. A study using a MEMS to assess adherence in common rheumatologic diseases recruited 29 gout patients and measured combined medication usage in relation to the total number of prescribed doses. The mean medication usage was 84% [15]. A study in South Korea employing pill counts in 129 gout patients found an adherence rate of 71% and persistence of 61% [16]. Unfortunately, these studies are small and of limited generalizability. Additionally, willingness to participate in a study with such close monitoring likely biases toward individuals more likely to adhere to medications.

RISK FACTORS FOR NONADHERENCE

Several recognized factors underlie risk for nonadherence to ULT. Older age and hypertension have

Table 1. Studies measuring adherence to urate lowering therapy

Reference	Site	Source	Size of gout population	Adherence at 1 year (%)	Authors
[11]	Israel	Healthcare organization database	7644	17	Zandman-Goddard *et al.*
[12]	United States	Administrative claims-based data	5597	18*	Riedel at al.
[13]	Italy	Research database	3727	3.2	Mantarro *et al.*
[14]	Ireland	Pharmacy claims-based records	15 908	35.3	McGowan *et al.*
[15]	Netherlands	MEMS	29	84**	de Klerk *et al.*
[16]	South Korea	Pill counts	132	71.2	Lee *et al.*
[17]	United States	Healthcare organization database	10 991	42	Rashid *et al.*
[22]	United Kingdom	Medical record database	49 395	39.7	Kuo *et al.*
[28]	New Zealand	Pharmacy records	953	78	Horsburg *et al.*
[30]	United States	Pharmacy filling records	9823	36	Solomon *et al.*

MEMS, Medication Event Monitoring Systems.
*'Compliance rate' measured as: days' supply from 1st prescription filled / [fill date of 2nd prescription filled – fill date of 1st prescription filled] for each fill and for up to 24 months of fill history.
**Average 'taking compliance' across all patients measured as (total number of recorded medication events / total number of prescribed doses) × 100%.

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consistently correlated with better medication adherence [8,9]; such patients tend to have more experience managing chronic diseases and taking daily medications. Interestingly, concomitant diuretic use and congestive heart failure in one study were associated with better ULT adherence despite more difficulty achieving serum urate goal [17]. Among a cohort of gout patients in the US Department of Veterans Affairs healthcare system, a cross-sectional analysis identified multiple comorbid conditions that complicated the choice of medication for treating acute gout flares and reduce serum urate. Many physicians prescribed specific gout therapies (including colchicine, NSAIDS, glucocorticoids, allopurinol, and probenecid) despite contraindications to the individual medications, raising the potential for noncompliance due to intolerance or adverse events when an alternative medication may have been better tolerated [18].

A disconnect between patient and provider perspective is another potential factor in treatment failure. Parties can complete a visit with different understandings and assumptions, often to the detriment of the patient. One study identified that providers were confident in their treatment of acute gout, their patients' adherence to ULT, and their own educational skills. Patients, however, felt that they lacked information, particularly about the role of ULT in gout management [19].

The achievement of serum urate targets across populations remain elusive partly because the complexities of gout treatment are underappreciated [20,21,25]. The concept that intercritical gout is associated with persistent low-level inflammation and asymptomatic monosodium urate (MSU) deposits, and is not a true remission, is one such example. Additionally, knowledge about the diverse array of gout medications is essential for avoiding adverse effects and managing patient expectations. In fact, lack of proper education may lead to loss of patient trust in both their physician and medication(s) [22,23].

PHYSICIAN MANAGEMENT OF GOUT: IMPACT ON ADHERENCE

The 'Management of Gout in the United States: A Claims-based Analysis' study employed administrative claims data to assess gout-related care. This study found that serum urate testing was performed in less than 70% of patients with chronic gout, and less than 60% of those with acute gout. Furthermore, less than 80% of patients with advanced gout (i.e., nontophaceous chronic, tophaceous chronic, or uncontrolled gout) received ULT, and on average, prescriptions for ULT covered less than half of the study year. Even a single rheumatology visit during the study period was associated with increased frequency of serum urate testing, increased number of ULT prescriptions, and reduced emergency department visits [24▪▪]. A UK study found that only 37% of patients received ULT, and only 18% of the prescriptions were started within six months of diagnosis. Timely initiation of ULT and appropriate referral to a specialist were areas of improvement identified for general practitioners [25]. These and other data suggest that, even before the question of adherence is considered, patients with gout are highly susceptible to underprescribing of ULT.

Oderda *et al* [26]. studied physician adherence to American College of Rheumatology (ACR) guidelines and scored physicians from 0 to 8 based on the categories of targeting a serum urate goal, first-line ULT prescribing, appropriate medication dosing, and gout flare prophylaxis while on ULT. Reviewing treatments of 350 chronic gout patients and their physicians' perspectives, rheumatologists tended to follow ACR guidelines more closely ($5.8/8 \pm 1.7$ versus $4.3/8 \pm 1.7$) and were more likely to be classified as 'higher adherence' (64.7 versus 46.3%) than primary care providers. There was a disparity between providers' belief that their patient care followed guidelines and their actual practice [26]. In one survey of real-world US practice patterns, a chart audit was conducted for 124 primary care physicians and 125 rheumatologists; out of 1245 gout patients' charts reviewed, only 11% achieved disease control (defined as an average serum urate ≤ 6, no flares, and no tophi) [21].

DIFFERENCES ACROSS POPULATIONS

Trends in ULT adherence and practice patterns are usually consistent across countries; however, healthcare disparities can further diminish care in subpopulations. Lower socioeconomic status and fewer clinic visits have been associated with worse compliance [11,27]. More specifically, Singh *et al.* [27] found a linear correlation between more outpatient visits and higher odds of reaching goal serum urate as well as less frequent allopurinol discontinuation.

A New Zealand prescription data analysis described worse ULT adherence among native Māori populations at 62% compared with 82% of non-Māori, highlighting the impact of potential healthcare disparities in gout [28]. Another community-based study addressing gout care, which included a Māori indigenous population, sent out an informational packet with material reflecting the current guidelines to a rural medical center. In comparing practice patterns before and after this educational package, provider education resulted in greater

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conformity to gout guidelines, and a higher percentage of patients achieving target serum urate [29▪].

Solomon *et al.* [30] assessed adherence in an elderly population enrolled in a pharmacy assistance program in Pennsylvania. The overall adherence rate was 36%, but Black individuals were twice as likely to be nonadherent [odds ratio (OR) 1.86, 95% confidence interval (CI) 1.52–2.27] compared with white individuals [30]. In another study exploring gout care in Black patients, patients were separated into high and low-adherence groups to assess differences in qualitative factors. Lack of side-effects from ULT, trust in physicians, and gout-related health literacy facilitated high adherence. Techniques for better adherence focused on incorporating medications into daily routines and using pillboxes. Suboptimal adherence was attributed to concerns about medication costs and side-effects, preference for alternative medicines, forgetfulness in both taking and refilling medications, lack of belief in ULT effectiveness, experience of side-effects, pill fatigue, concern for drug interactions, pill size, and other competing priorities [31].

GOUT GUIDELINES

In 2020, three professional organizations released updated guidelines for gout management. Treat-to-target remains a strong recommendation from all rheumatologic societies. The ACR recommends ULT and maintaining serum urate less than 6 mg/dl over no target [32▪]. There is moderate-to-high-quality evidence for the treat-to-target approach as evidenced by target-driven protocols resulting in better adherence to ULT, fewer gout flares, and reduction in size of tophi [32▪,33▪▪,34▪,35▪▪]. The main pillars of this recommendation are ULT dose titration and frequent assessment of serum urate levels to guide therapy.

The Japanese guideline had already established a serum urate target of less than 6.0 mg/dl in 2011, citing the 'dissolution limit of uric acid in the body fluid' at 6.4 mg/dl, and went on in 2020 to recommend this goal for tophaceous gout [36,37▪]. The French Society for Rheumatology echoes the recommendation for a target serum urate less than 6.0 mg/dl and goes further, recommending a target less than 5.0 mg/dl whenever possible [38]. These various guidelines underscore the importance of maintaining serum urate at goal and monitoring serum urate levels regularly because MSU crystals can take years to dissolve. Dual-energy CT studies have shown that MSU crystal burden is reduced after reaching serum urate goal but MSU deposits may persist beyond two years of serum urate control [39,40].

The American College of Physician's controversial guideline to 'treat to avoid symptoms' avers that insufficient evidence supports the benefits of ULT treatment and/or a treat-to-target approach [41]. The dissonance between the rheumatology and internal medicine society guidelines leads to provider confusion, especially as most gout patients, including newly diagnosed cases, are managed by primary care providers.

INTERVENTIONS TO IMPROVE ADHERENCE

Accumulating evidence supports the utility of including nonphysician providers in gout care as opposed to primary care or rheumatology alone. Such individuals can supplement physician caregivers, provide patient education, regularize follow-up and, in contrast to primary care physicians, focus exclusively on the gout treatment. The ACR recommends that nonphysician providers use a protocolized approach while incorporating the key elements of patient education and shared decision-making [32▪]. Table 2 summarizes the studies that assess the effect of various interventions on adherence to ULT.

One approach to increasing patient engagement is using mobile apps. In a study among a New Zealand gout population, patients were randomized to use a commercially available 'Gout Central' app, versus a dietary app as a control (DASH diet app). The primary outcome was user engagement. Important features of the gout app included a serum urate tracker, a gout flare tracker, an educational section, and a reminder-based function. The 36 users of the gout app were more engaged and reported increased gout-related knowledge, including awareness of adverse long-term consequences of gout and hyperuricemia. However, the two groups did not differ in terms of behavioral changes at the end of the two-week study period [42▪]. Major limitations of this study were its small sample size, short duration, and the lack of utility of the app for those with infrequent flares. App development and technological advances more broadly may offer the prospect of better patient engagement and closer serum urate monitoring.

Mikuls *et al* [33▪▪]. reported a study comparing a pharmacist-led gout treatment protocol versus usual care among 1462 patients who were initiating allopurinol for gout (RAmP-UP Study). The protocol utilized automated interactive telephone-based interventions, which provided reminders and encouragement, and checked adherence. On identification of nonadherence, pharmacists would call patients as a 'step-up' in intervention. This approach resulted in significantly improved allopurinol adherence compared with usual care (50 versus 37%; OR 1.68; 95% CI 1.30, 2.17). Additionally, the serum

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Table 2. Effectiveness of interventional trials

Trial (reference)	Site	Study size	Intervention	Duration	Primary outcome	Effect of intervention
Nottingham Gout Treatment Trial [35▪▪]	United Kingdom	517	Nurse-led protocolized care	2 years	Serum urate < 6.0 mg/dl	Nurse-led versus usual: 95 versus 30% (RR 3.18, 95% CI 2.42–4.18, $P < 0.0001$)
RAmP-Up Trial [33▪▪]	United States	1463	Pharmacist-led protocolized care incorporating automated telephone screening	1 year of intervention followed by 1 year of monitoring only	Allopurinol adherence and serum urate < 6.0 mg/dl at 1 year	Adherence, protocol versus usual: 50 versus 37% (OR 1.68; 95% CI 1.30, 2.17) Serum urate at goal, protocol versus control: 30 versus 15% (OR 2.37; 95% CI 1.83, 3.05)
Pharmacist-managed titration [34▪]	Unites States	47	Pharmacist-led protocolized care	12 months	Serum urate < 6.0 mg/dl	Intervention versus control: 32 versus 25%
Mobile Health App [42▪]	New Zealand	72	Commercially available gout self-management app	2 weeks	User version of the Mobile Application Rating Scale	More engaging than comparator app (mean difference −0.58, 95% CI −0.96 to −0.21)

CI, Confidence interval.

urate goal was more likely to be achieved (30 versus 15%; OR 2.37; 95% CI 1.83, 3.05). A similar study involving a smaller group of patients reported that pharmacist-managed titration of ULT achieved target serum urate in 32% of patients compared with 25% of patients under standard care [34▪]. Although these two studies highlight that fewer than one-third of patients in the interventional groups achieved serum urate goal, they still represent significant improvements over standard care. This highlights that guideline-driven protocols are not enough and that patient cooperation toward a common goal is integral in gout care.

A British study compared a nurse-led gout protocol with usual physician care and achieved target serum urate in 95% of patients in the nurse-led group at two years. The Nottingham Gout Treatment Trial thus demonstrated the most effective intervention for gout adherence of any study to date. For 255 of the 517 study patients, nurses followed treat-to-target guidelines while utilizing education and shared decision making as the cornerstones to gout care. The nurse-led arm had improved outcomes across the board including a persistence rate of more than 95%, achievement of serum urate of less than 5 mg/dl in 88% of patients, a reduction in flare frequency, a reduction in all tophi parameters (size, number, and presence), and improved quality of life measures. A posthoc analysis reiterated the relationship between increased serum urate and an increased frequency of gout flares. The report goes on to cite a cost per quality-adjusted life year of £5066 (~$6500), with greater projected cost reductions the longer the intervention continued. Once achieved, good gout control could permit fewer provider visits and less frequent monitoring [35▪▪]. Other takeaways from this study include the fact that usual care was unsatisfactory, leading to virtually no change in serum urate over two years. Furthermore, the study proved that following professional guidelines can result in near complete response for chronic gout. Lastly, regardless of the provider, time spent engaging the patient regularly achieves results. A follow-up study used questionnaires to assess patient perspectives and perceptions after trail completion. In the 82% of participants who returned questionnaires, there was a preference toward protocolized care. The intervention led to improved gout-related knowledge, more willingness to take ULT, and fewer patient-reported flares [43▪▪].

One difference between this nurse-led protocol and the pharmacist and app studies described above was that the nurse study involved multiple face-to-face visits, whereas the others took a telehealth approach. Whether this or other factors led to the remarkable success of the nurse study remains to be determined. The nurse-led study could have had a sampling bias toward participants who were willing to accept frequent outpatient visits, therefore selecting for those motivated to actively participate in their gout care. Moreover, the extent to which a nurse-led approach would translate to other cultural environments needs to be evaluated.

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In the era of the Coronoa virus disease 2019 (COVID-19) pandemic, access to in-person care is more limited and telehealth will be integrated into clinical practice for the foreseeable future. Adapting to these challenges will be critical to gout care when face-to-face interactions are limited and in a future which technology is more widely employed. Guideline-driven care and patient engagement should be the guiding principles whether the focus will turn to app-based interventions or increasing utilization of telehealth.

CONCLUSION

Nonadherence remains an important barrier to optimal gout care. Modifiable adherence can be separated into patient adherence to ULT and prescriber adherence to guidelines. As most patients with gout are treated by primary care providers, the question of which guidelines (if any) providers follow remains a concern. A start to addressing factors underlying nonadherence would incorporate treat-to-target protocols for gout patient care. Trained nonphysician providers could be integrated into the primary care setting and consult with rheumatology as needed. More innovative approaches are needed to address inadequacies in gout care.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

None. For the purposes of full transparency, M.H.P. reports that he is the recipient of investigator-initiated grants from Hikma Pharmaceuticals and Horizon Therapeutics.

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The role of diet in hyperuricemia and gout

Chio Yokose[a,b], *Natalie McCormick*[a,b,c], *and Hyon K. Choi*[a,b]

Purpose of review
Although gout's cardinal feature is inflammatory arthritis, it is closely associated with insulin resistance and considered a manifestation of the metabolic syndrome. As such, both gout and hyperuricemia are often associated with major cardiometabolic and renal comorbidities that drive the persistently elevated premature mortality rates among gout patients. To that end, conventional low-purine (i.e., low-protein) dietary advice given to many patients with gout warrant reconsideration.

Recent findings
Recent research suggests that several healthy diets, such as the Mediterranean or Dietary Approaches to Stop Hypertension (DASH) diets, in combination with weight loss for those who are overweight or obese, can drastically improve cardiometabolic risk factors and outcomes. By treating gout as a part of the metabolic syndrome and shifting our dietary recommendations to these healthy dietary patterns, the beneficial effects on gout endpoints should naturally follow for the majority of typical gout cases, mediated through changes in insulin resistance.

Summary
Dietary recommendations for the management of hyperuricemia and gout should be approached holistically, taking into consideration its associated cardiometabolic comorbidities. Several healthy dietary patterns, many with similar themes, can be tailored to suit comorbidity profiles and personal preferences.

Keywords
diet, gout, hyperuricemia, metabolic syndrome, nutrition

INTRODUCTION

Although gout's cardinal feature is inflammatory arthritis, its primary underlying cause, hyperuricemia, is considered a manifestation of the metabolic syndrome mediated by insulin resistance [1,2]. Thus, hyperuricemia and gout are both associated with adverse consequences of the metabolic syndrome, namely cardiometabolic and renal disease [3–5]. To that end, the conventional low-purine (i.e., low-protein) dietary approach focusing on prevention of purine-loading can worsen its cardiometabolic comorbidities by leading to compensatory higher consumption of carbohydrates (including fructose) and fats (including trans or saturated fat). Moreover, the long-term effectiveness of a low-purine diet to lower urate levels remains unclear with its limited palatability and sustainability [1,6]. In contrast, there are several preeminent healthy diets that can simultaneously reduce serum urate and the risk of gout and overall cardiometabolic risk by lowering adiposity and insulin resistance. These dietary interventions can be applicable to patients at all stages of gout (Fig. 1) and should form a cornerstone of lifestyle counseling for such patients. This article reviews the relevant scientific rationale and available data to provide evidence-based dietary considerations for the prevention and management of hyperuricemia and gout, including the role of diet on gout flares, together with its cardiometabolic comorbidities holistically.

THE METABOLIC SYNDROME AND COMORBIDITIES OF HYPERURICEMIA AND GOUT

Insulin resistance is a key feature of the metabolic syndrome, and because insulin resistance can reduce the renal excretion of urate [6–9], hyperuricemia and

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Curr Opin Rheumatol 2021, 33:135–144
DOI:10.1097/BOR.0000000000000779

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KEY POINTS

- Gout and hyperuricemia can be considered components of the metabolic syndrome, as insulin resistance leads to renal underexcretion of uric acid.
- Gout and hyperuricemia are strongly associated with cardiometabolic comorbidities that drive the persistently elevated premature mortality observed among those with gout.
- Therefore, dietary interventions that target metabolic syndrome should have the dual, synergistic effect of improving cardiometabolic risk factors while reducing the risk of gout.
- Conventional guidance to follow a low-purine (i.e., low-protein) diet may be detrimental as it may lead to the increased consumption of (often refined) carbohydrates and fats (including saturated and trans fats) which can worsen gout's cardiometabolic comorbidities and may actually contribute to hyperuricemia and gout risk.
- There are several healthy dietary patterns, including the Mediterranean and DASH diets, which have been investigated in interventional or observational cohort studies and found to be beneficial both for cardiometabolic risk mitigation as well as gout and hyperuricemia.

gout closely coexist with metabolic syndrome [9,10]. In the US general population, the prevalence of metabolic syndrome has exceeded more than 71% among gout patients aged 40 years and older [10], compared with an overall prevalence of 22% among all US adults in the same time period [11]. Similarly, the prevalence of the metabolic syndrome tends to rise progressively with increasing serum urate levels, reaching as high as nearly 90% among women with serum urate level more than 10 mg/dl [12]. It is well recognized that patients with gout shoulder a high burden of related comorbidities, including hypertension (74%), chronic kidney disease stage 2 and above (71%), obesity (53%), diabetes (26%), myocardial infarction (14%), and heart failure (11%) [3], which are 2–3 times more prevalent in those with gout compared with those without [3].

In addition to these cross-sectional associations, patients with gout are at an increased risk of future cardiometabolic complications. Gout is associated with a 41% increased risk of incident type 2 diabetes [13], 33% increased risk of peripheral arterial disease [14], and 60% increased risk of coronary heart disease (CHD) among men [15]. Consequently, individuals with gout are also at increased risk of myocardial infarction [16] and all-cause and cardiovascular disease (CVD) mortality [17,18]. Thus, any dietary recommendation to address hyperuricemia and gout should simultaneously provide cardiometabolic benefits to reduce this excess morbidity and mortality.

Failure to do so effectively in current practice appears to be reflected in a general population-based cohort study that showed that the level of premature mortality among gout patients remained unimproved over the past two decades (Fig. 2a) [19], unlike rheumatoid arthritis [20], where mortality improved substantially during the same period (Fig. 2b). Furthermore, two recent analyses on the global burden of disease have reported increases in age-adjusted prevalence and disability-adjusted life-years of gout every year from 1990 to 2017 and have identified intensive dietary management as a possible strategy to reverse this trend [21▪▪,22▪▪].

CAUSAL PATHWAYS FOR OBESITY, HYPERURICEMIA, AND GOUT

The rising prevalence of gout [23] closely following the obesity epidemic in the United States and other Westernized nations can be explained by changes in diet (including larger portion sizes [24] and

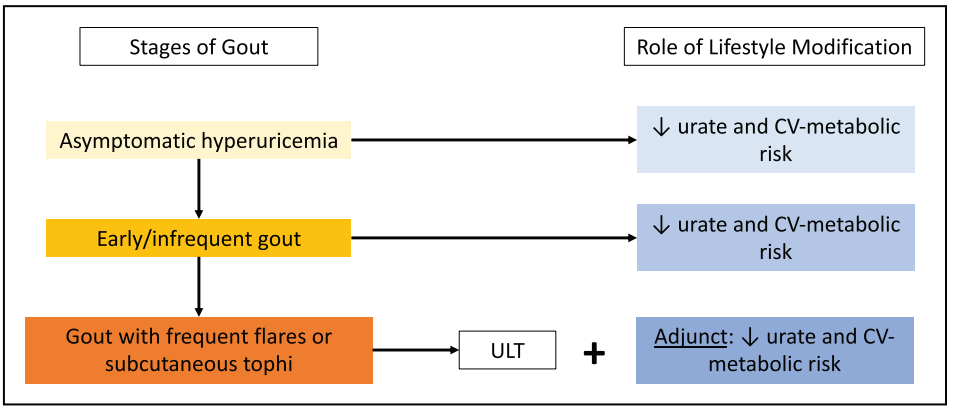

FIGURE 1. Stages of gout and role of lifestyle interventions. CV, Cardiovascular; ULT, urate-lowering therapy.

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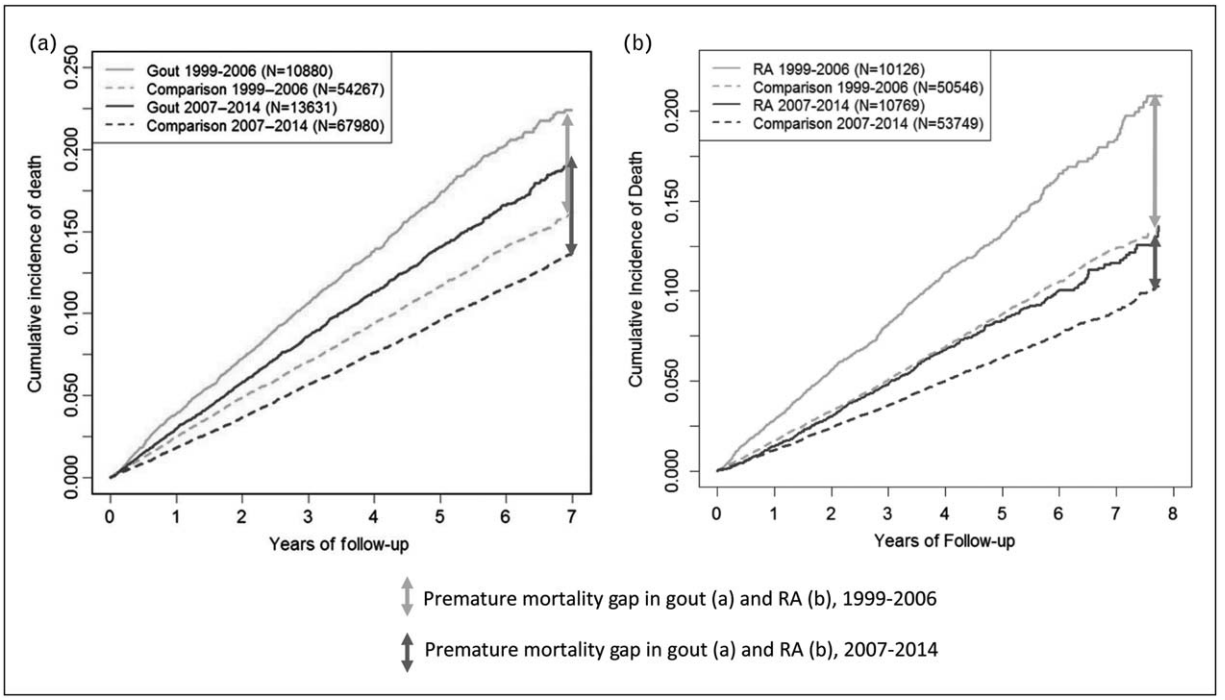

FIGURE 2. Persistent premature mortality among patients with gout remains higher than premature mortality in rheumatoid arthritis in recent decades. Panel (a) compares the cumulative incidence of death from 1999 to 2006 (lines) to that from 2007 to 2014 (bottom lines) among patients with gout (solid lines) and without gout (dotted lines), with the difference between the solid and dotted lines remaining unchanged during the two time periods, indicating persistent premature mortality. Conversely, panel (b) compares the cumulative incidence of death from 1999 to 2006 (top lines) to that from 2007 to 2014 bottom lines) among patients with rheumatoid arthritis (solid lines) and without rheumatoid arthritis (dotted lines). The difference in mortality between the two blue lines is substantially smaller than that between the two red lines, indicating an improvement in the mortality gap among patients with rheumatoid arthritis in the latter time period. Adapted from [19,20]. RA, Rheumatoid arthritis.

unhealthy dietary composition) and sedentary lifestyle [25–27,28▪▪,29,30]. Although diet quality (i.e., isocaloric composition pattern without impacting BMI, or direct effect in Fig. 3) has been the main focus in gout care (e.g., low-purine diet), it is important to recognize the impact of diet quantity (caloric intake) and physical activity (caloric output) on the risk of obesity as well as the subsequent risk of cardiometabolic endpoints, including hyperuricemia and gout (indirect effect mediated by obesity, Fig. 3) [6,31▪]. To this end, a recent analysis of the US general population found BMI was the most

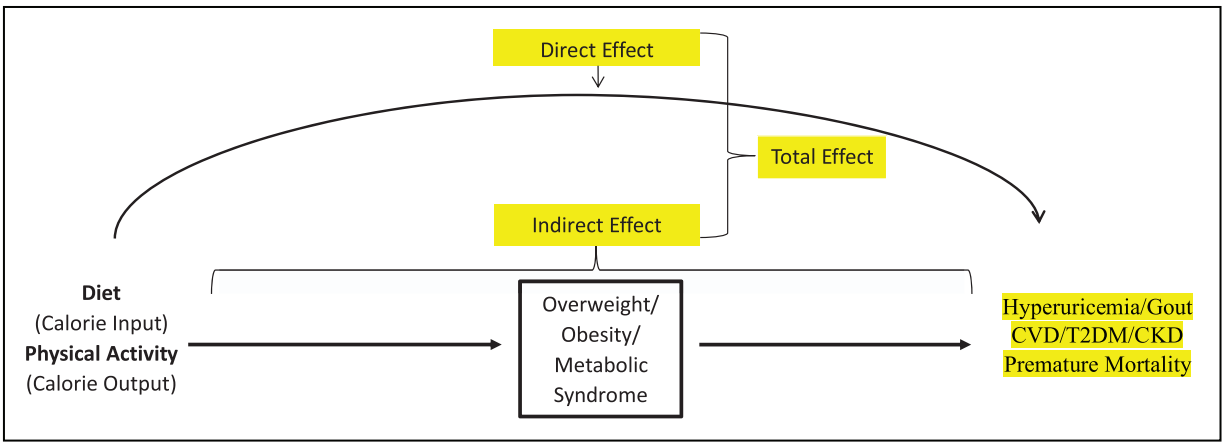

FIGURE 3. Causal pathway linking lifestyle factors with gout and cardiovascular-metabolic disease. CKD, Chronic kidney disease; CVD, cardiovascular disease; T2DM, type 2 diabetes mellitus.

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important modifiable risk factor for hyperuricemia, with a population attributable risk (i.e., the proportion of hyperuricemia cases attributable to overweight or obesity) of 44% [28▪▪].

SHARED RISK FACTORS AND HEALTHY EATING PYRAMID

A series of prospective investigations and ancillary studies have investigated the risk of gout associated with relevant lifestyle factors, many of which can be overlaid on a Healthy Eating Pyramid that is designed to prevent major conditions such as CVD and type 2 diabetes (Fig. 4) [7,8,32]. As such, nutritional advice for both cardiometabolic disease and gout centers around weight control and adherence to a general dietary pattern that emphasizes whole grains, healthy unsaturated oils, vegetables and fruits, nuts and legumes, and healthy protein such as poultry, fish, eggs, and low-fat dairy, while limiting the consumption of red meat, refined carbohydrates, and saturated fats. This framework is the repeated theme of healthy diets recommended by the American Heart Association (AHA)/American College of Cardiology (ACC) [33] and Dietary Guidelines for Americans 2015–2020 [34], discussed in detail under cardiometabolic diets below.

LIMITATIONS OF LOW-PURINE DIET

The current conventional lifestyle approach for gout focuses on limitation of protein to reduce purine-loading [1]. When the intake of one macronutrient is reduced (e.g., protein), this must be accompanied by a compensatory increase in one or both of the remaining macronutrients (e.g., carbohydrates and fats). Given the prevalence of Western-style diets and deterioration of healthy eating habits [35], there is the risk of protein-restriction leading to increased consumption of foods that are rich in refined carbohydrates (including fructose) and saturated or trans fats. These changes could further exacerbate insulin resistance, leading to higher plasma levels of glucose and lipids, thereby contributing to the development and worsening of metabolic syndrome and its complications in patients with gout [1,6]. Furthermore, the long-term therapeutic value of a purine-restricted diet has been questioned because of limited palatability, sustainability, and antigout efficacy [1,6].

HEALTHY CARDIOMETABOLIC DIETS

In contrast, approaches that focus on comprehensive healthy dietary patterns to reduce insulin resistance may be preferable for patients with gout and

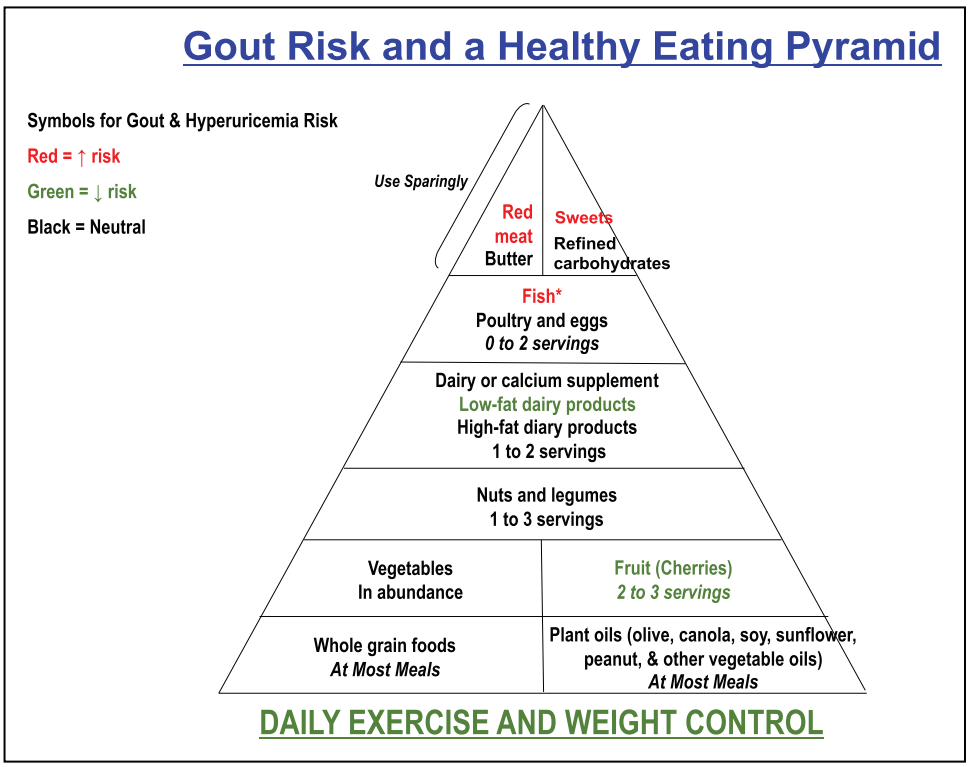

FIGURE 4. Evidence-based healthy eating pyramid for gout. *Fish is the only exception where recommendations for gout (short term) and cardiometabolic health may be contradictory. In the long-term, patients with gout would still benefit from moderate fish consumption if their gout/hyperuricemia is controlled by other measures.

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hyperuricemia to simultaneously address both gout and cardiometabolic risk factors. Based on interventional and prospective cohort studies, several dietary patterns have emerged as preeminent approaches for cardiometabolic health, including the Mediterranean (https://www.hsph.harvard.edu/nutritionsource/healthy-weight/diet-reviews/mediterranean-diet/) and dietary approaches to stop hypertension (DASH) (https://www.hsph.harvard.edu/nutritionsource/healthy-weight/diet-reviews/dash-diet/) diets. These diets incorporate many aspects of the aforementioned Healthy Eating Pyramid and are endorsed by both the AHA/ACC [33] and Dietary Guidelines for Americans 2015–2020 (https://health.gov/our-work/food-nutrition/2015–2020-dietary-guidelines/guidelines/) [36]. Below we discuss in detail the benefits of the Mediterranean and DASH diets among patients with gout. We also summarize several studies which have evaluated the benefits of weight loss through dietary interventions on the effect of gout and cardiometabolic endpoints, which supports adiposity and insulin resistance as being key targets for lifestyle intervention.

The Mediterranean diet

The Mediterranean diet consists of a high intake of monounsaturated fat (primarily from olive oil), plant proteins, whole grains, and fish, accompanied by moderate intake of alcohol and low consumption of red meat, refined grains, and sweets [37], resembling the Healthy Eating Pyramid (Fig. 4). The Mediterranean diet has been shown to reduce the risk of CVD events and cardiovascular mortality [38–45], including a 73% lower rate of coronary events and a 70% lower rate of total mortality, compared with a usual postinfarct 'prudent' diet in secondary prevention [45]. Furthermore, two variations of the Mediterranean diet (one supplemented with olive oil, the other with nuts) were both associated with a more than 50% lower risk of incident type 2 diabetes compared with a control diet [46]; findings to this effect have also been replicated in observational cohort studies including the Health Professionals Follow-Up Study [47]. In randomized dietary weight loss trials, adherence rates to the Mediterranean diet have been high [38], reaching 85% [48] in one, suggesting that such a dietary strategy may be more sustainable than the conventional low-purine diet currently recommended for patients with gout.

Several studies have investigated the beneficial serum urate and gout effects of the Mediterranean diet. For instance, an ancillary analysis of the Prevención con Dieta Mediterránea trial showed that participants in the highest quintile of adherence to the Mediterranean diet had 23% lower odds of having hyperuricemia compared with those in the lowest quintile [49]. Furthermore, in a secondary analysis of one of the aforementioned dietary weight loss trials [48], the Mediterranean diet with calorie restriction resulted in a mean reduction in serum urate from baseline of 0.8 mg/dl for all participants and 2.1 mg/dl among those with baseline hyperuricemia (serum urate $\geq$7 mg/dl) [31▪].

The Dietary Approaches to Stop Hypertension diet

The DASH diet, which emphasizes whole grains, fruits, vegetables, and low-fat dairy products, with a high intake of plant protein from legumes and nuts in lieu of animal protein sources, was initially developed and studied for the management of hypertension [51–53]. The original DASH and DASH-Sodium (DASH with reduced sodium intake) diets have been shown to significantly lower both systolic and diastolic blood pressure as well as total and LDL cholesterol [52,53]. The OmniHeart trial, which compared a traditional DASH diet with a modified DASH diet with partial substitution of carbohydrates for either healthy sources of protein or monounsaturated fats, showed that in addition to the blood pressure benefits seen with all three diets, the protein-rich and unsaturated fat-rich diets resulted in a significantly greater increase in HDL and decrease in triglycerides. The protein-rich diet also showed a significantly greater decrease in LDL relative to the traditional DASH diet [54]. A recent ancillary analysis of this study looking at serum urate endpoints reported that the protein-rich diet reduced serum urate from baseline to the end of the six-week feeding period more than the carbohydrate-rich or unsaturated fat-rich diets (mean change of −0.12 mg/dl [95% confidence interval (CI), −0.23 to −0.02] for the protein-rich diet, compared with 0 mg/dl for both carbohydrate-rich and unsaturated fat-rich diets). However, all three diets did significantly reduce serum urate among those with baseline hyperuricemia (serum urate $\geq$6 mg/dl), (all $P \leq 0.003$) with no between-group differences [55]. These results are consistent with the notion that a protein-restricted diet may not necessarily be the best option for patients with gout.

Furthermore, several interventional and observational cohorts have reported on the benefits of the DASH diet in reducing the risk of CVD [43,56,57], type 2 diabetes [47,58], and mortality [44,59–61]. For example, in the Nurses' Health Study, those with the highest quintile DASH scores had a 24% lower risk of incident CHD and 29% lower risk of CHD mortality compared with those with the lowest quintile DASH scores [57]. Based on this evidence,

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the original DASH diets, as well as the modifications studied in the OmniHeart trial, have also been endorsed by both the ACC/AHA [33] and Dietary Guidelines for Americans 2015–2020 [34] as another healthy dietary option. Additionally, this suggests that within the framework of the Healthy Eating Pyramid, dietary modifications based on comorbidities and personal preferences are possible without diminishing the beneficial effects of these diets.

As hypertension is present in 74% of patients with gout (and in 50% of people with hyperuricemia) [3], it can be argued that the DASH diet would already be indicated for the majority of gout patients to manage their hypertension. Nevertheless, an ancillary analysis of the DASH-Sodium trial showed that the DASH diet resulted in a reduction in serum urate of 0.35 mg/dl compared with controls; in a subgroup analysis, the reduction in serum urate was more pronounced among those with baseline hyperuricemia, with a reduction of 0.76 and 1.29 mg/dl among those with serum urate 6–7 and at least 7 mg/dl, respectively [62]. Similarly, an ancillary analysis of a study that involved the partial replacement of a typical diet with the DASH diet suggested a trend toward greater serum urate reduction among African-American participants with baseline hyperuricemia [63]. In a large population-based cohort of Chinese adults [64], the highest quartile DASH diet score was associated with 30% lower odds of hyperuricemia cross-sectionally. Importantly, this association was significantly greater among physically inactive adults (odds ratio 0.56, 95% CI, 0.50–0.63) than those with moderate or high levels of physical activity (odds ratio 0.86, 95% CI, 0.78–0.95; P for interaction = 0.008) [64]. Additionally, an analysis of the Health Professionals Follow-Up Study revealed that men with the highest quintile DASH scores had a 32% lower risk of incident gout compared with those with the lowest quintile DASH scores [65]. The same analysis among women in the Nurses' Health Study revealed a similar risk reduction for incident gout with the DASH diet [50].

In many ways, a DASH diet shares a number of similarities with a vegan or vegetarian diet, which too have been associated with weight loss and improved cardiometabolic health [66,67]. Accordingly, evidence from two nonrandomized longitudinal cohort studies suggests that vegetarian diets may also decrease the risk of incident gout [68], with fully adjusted odds ratios of 0.40 (95% CI, 0.17–0.97) and 0.61 (95% CI, 0.41–0.88), for vegetarians compared with nonvegetarians. However, a recent analysis comparing DASH, fruit and vegetable, and control diets revealed that, among those with baseline hyperuricemia, the DASH diet lowered serum urate levels more robustly than the control diet, whereas the serum urate-lowering effect of the fruit and vegetable diet was of only borderline significance [69▪]. These results suggest that while increasing fruit and vegetable consumption is a key feature of the DASH diet, there are additional benefits to be gleaned from the dietary pattern as a whole, as opposed to emphasizing a few healthy food groups.

Effects of weight loss diets

Beyond the benefits of isocaloric diets intended to maintain stable weight, reducing insulin resistance through weight loss in overweight and obese individuals could improve both gout and associated cardiometabolic risk. For example, in an ancillary analysis of the Dietary Intervention Randomized Controlled Trial (DIRECT) [48], three diets (low-fat, restricted-calorie; Mediterranean, restricted-calorie; low-carbohydrate, nonrestricted-calorie, modeled after the Atkins diet) all resulted in a mean reduction in serum urate from baseline of 0.8 mg/dl over six months. The effects were more pronounced among those with baseline hyperuricemia (serum urate $\geq$7 mg/dl), with a serum urate reduction from baseline ranging from 1.9 mg/dl with the low-fat diet to 2.4 mg/dl with the low-carb diet (Fig. 5) [31▪]. Over six months, all three diets resulted in significant weight loss ranging from 5.0 kg with the low-fat diet to 7.0 kg with the low-carb diet, as well as improvements in other cardiometabolic parameters such as lipids and fasting insulin levels [31▪]. In this secondary analysis, the serum urate reduction attenuated at 24 months (ranging from 1.1 to 1.4 mg/dl reduction among those with baseline hyperuricemia), likely mediated by regaining some of the weight that had been lost during the initial six months of the study [31▪].

Furthermore, a pilot study (n = 13 gout patients) that aimed to lower insulin resistance over 16 weeks by reducing calorie intake with a diet high in protein (i.e., the opposite of the conventional low-purine diet) and low in carbohydrates and saturated fat found that mean serum urate levels decreased from 9.6 to 7.9 mg/dl and the frequency of monthly gout flares decreased from 2.1 to 0.6 [6]. Additional cardiometabolic benefits included significant improvements in total cholesterol, total cholesterol/HDL-C ratio, and triglyceride levels [6]. Together, these studies support that dietary approaches to reduce insulin resistance through weight loss in overweight and obese individuals could improve both gout outcomes and associated cardiometabolic risk. Further, these studies suggest that the conventional low-purine dietary advice given to many patients with gout warrants reconsideration.

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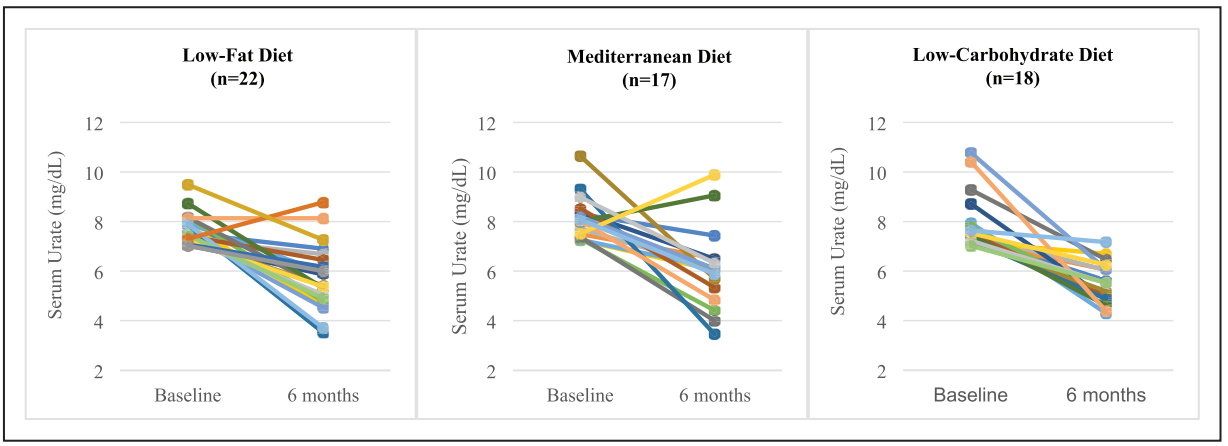

FIGURE 5. Serum urate change at six months among participants with baseline hyperuricemia in ancillary analysis of dietary intervention randomized controlled trial. Adapted from [31▪].

PERSONALIZED LIFESTYLE RECOMMENDATIONS

Given the multiple healthy dietary patterns to choose from, the particular diet that is adopted by a given individual should be guided by their concurrent comorbidities and personal preferences. To aid in the personalization of these lifestyle recommendations, there are ongoing efforts to identify phenotypically distinct clusters, or subtypes, of gout based on comorbidities. For example, Richette *et al.* [70] performed a comorbidity cluster analysis among a cohort of French patients with gout, and identified five distinct subtypes of gout as follows: isolated gout with few comorbidities, obesity with high prevalence of hypertension, type 2 diabetes, dyslipidemia, and cardiorenal disease. Similar analyses have been performed among a prospective gout cohort in the United Kingdom and using the nationally representative NHANES data in the United States [71,72]. Although the generalizability of these comorbidity clusters remains to be elucidated, these data suggest that personalized lifestyle counseling may be possible for patients with gout to identify the most appropriate interventions for their comorbidities and preferences (Fig. 6). For example, the DASH diet may be ideal for patients with hypertension and can be implemented with calorie restriction for those who are overweight or obese. For patients who have hypertension but have a preference for more protein in their diet, the protein-enriched

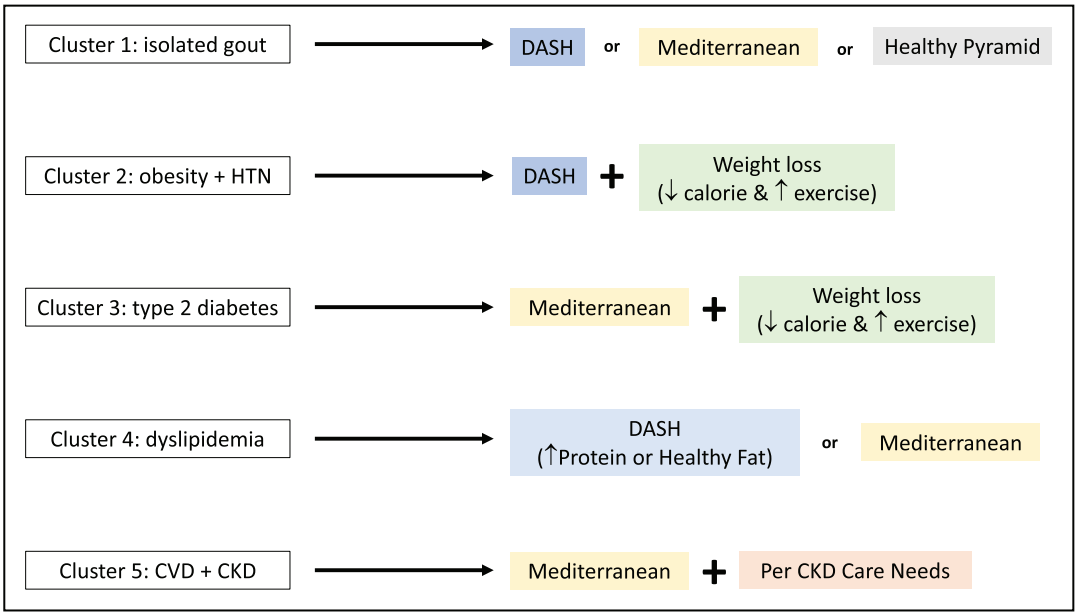

FIGURE 6. Potential personalized approaches based on comorbidity cluster profiles.

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DASH diet from the OmniHeart trial [54] could lead to better long-term adherence. For patients who require better lipid or glycemic control, the Mediterranean diet may be most suitable based on improvements in HDL, triglycerides, and markers of insulin resistance [48].

IMPLICATIONS ON GOUT FLARES

Hyperuricemia is a prerequisite for developing gout and thus should be the target for the long-term management of gout; however, it is worthwhile to note the implications of diet on gout flares in the short term. For instance, short-term exposures to purine-rich foods have been associated with recurrent gout flares in a self-controlled, case-crossover study [73]. Many of the purine-rich foods assessed in this study included animal sources such as red meat and organ meats, which are to be used only sparingly according to the Healthy Eating Pyramid and cardiometabolic diets and thus, are best avoided regardless of whether one is using diet to mitigate the risk of recurrent gout flares or for long-term gout and cardiometabolic comorbidity management.

Interestingly, this case-crossover study found that consumption of high-purine foods of plant origin, such as peas, lentils, spinach, and asparagus, was not significantly associated with increased risk of recurrent gout flares [73]. Again, this is compatible with the recommendations of the healthy cardiometabolic diets such as the Mediterranean and DASH diets to regularly consume legumes and vegetables. One purine-rich food item for which short and long-term recommendations may be seemingly contradictory is seafood, which is a notable feature of the healthy cardiometabolic diets, and are a good source of ω-3 fatty acids (especially fatty fish) and lean protein (both fish and shellfish) [36]. In an Internet-based case-crossover study, ω-3 fatty acids were associated with a reduced risk of recurrent gout flares [74], but excessive seafood consumption may nevertheless be associated with short-term increased risk of recurrent gout flares in the context of its purine content [73]. Thus, it may be advisable to limit the consumption of seafood in the short-term among patients who are having frequent gout flares or during the initial phase of urate-lowering drug therapy. However, allowing seafood back into the diet is likely overall beneficial with the use of prophylactic antiinflammatory agents such as colchicine (if needed) or once serum urate is sufficiently lowered through other means. With these maintenance measures in place, the long-term avoidance of seafood based solely on its potential to trigger recurrent gout flares (without compensatory consumption of other healthy proteins) is unadvisable, given its known cardiometabolic benefits. A similar paradigm might apply to allowing for light-to-moderate wine consumption, which has been associated with short-term increased risk of gout flares [75] but not identified as a risk for incident gout [76] or hyperuricemia [77]. Furthermore, a recent randomized trial of moderate wine consumption has suggested cardiometabolic benefits [78].

THE NEED FOR FURTHER RESEARCH

As summarized, the rationale behind the cardiometabolic diets and weight loss approaches are strong and existing clinical data are very supportive; yet, high-level evidence from randomized trials specifically among gout patients are limited to date. Large-scale clinical trials of Mediterranean or DASH diets with calorie restriction among gout patients, similar to the approaches of the DIRECT trial [48], are warranted, as is further research into the effects of plant-based diets on gout risk and outcomes. Furthermore, nutrition research that incorporates patient preference and improving adherence would be highly relevant given the well-known long-term challenges in sustaining these lifestyle interventions. A web-based educational tool called MyGoutCare, codeveloped by gout patients and clinical experts, was associated with improved patient knowledge in a pilot study [79], where it helped them identify actionable changes, including dietary changes; pilot data for GoutCare [80], a mobile application with an emphasis on diet and weight management, are also promising. Although longer term assessments of clinical endpoints are needed, these educational and self-management tools may assist patients in understanding and implementing the dietary recommendations outlined in this review.

CONCLUSION

In conclusion, several well-established healthy eating patterns, such as the Mediterranean and DASH diets, with or without calorie restriction to achieve weight loss, can all lower serum urate levels, although the effect size is smaller than that of a typical urate-lowering drug. Cardiometabolic risk factors, including BMI, blood pressure, cholesterol profile, triglycerides, and insulin resistance, also improve with these diets (consistent with their originally proven roles), whereas such nongout benefits remain unclear with urate-lowering drugs. Existing evidence suggests that the long-term adoption of a low-purine dietary approach for gout management is neither helpful nor sustainable for patients with gout and may have detrimental cardiometabolic consequences. A paradigm shift that considers gout

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as a part of the metabolic syndrome and focuses on comprehensive dietary patterns as opposed to singular food items is necessary. By focusing our dietary recommendations on dietary patterns which have been shown to reduce cardiometabolic risk factors, the beneficial effects on gout endpoints should naturally follow for the majority of typical gout cases, mediated through changes in insulin resistance. Although diet alone may not supplant the need for urate-lowering therapy among patients with gout, it is a powerful adjunctive tool to comprehensively address the cardiometabolic burden and premature mortality among patients with gout.

Acknowledgements

None.

Financial support and sponsorship

C.Y. is supported by T32 AR007258 and Rheumatology Research Foundation Scientist Development Award. N.M. is supported by a Fellowship Award from the Canadian Institutes of Health Research. H.C. is supported by R01 AR065944, P50 AR060772.

Conflicts of interest

C.Y. and N.M. have no conflicts of interest. H.K.C. reports consulting fees from Ironwood, Selecta, Horizon, Takeda, Kowa, Vaxart and research support from Ironwood, Horizon.

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Medications for gout and its comorbidities: mutual benefits?

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Purpose of review
To review recent literature with relevance to the management of multimorbid patients with gout, i.e., gout medication repurposed for comorbidities and vice versa.

Recent findings
Adding to the previous success of interleukin-1 inhibition, two trials on low-dose colchicine's role in cardiovascular disease (CVD) demonstrated potential benefits in patients with or without gout. In Colchicine Cardiovascular Outcomes Trial, a composite CVD endpoint was reduced by 23% among patients who had experienced a recent myocardial infarction. In Low-Dose Colchicine 2, the composite CVD endpoint was reduced 31% among those with stable coronary artery disease. Use of urate-lowering therapy (ULT) for renal protection in patients without gout produced null results. Allopurinol did not benefit the glomerular filtration rate in two trials (Controlled trial of slowing of Kidney Disease progression From the Inhibition of Xanthine oxidase and Preventing Early Renal Function Loss) among patients with chronic kidney disease (with or without hyperuricemia, but not gout). SGLT-2 inhibitors, a medication recommended for patients with diabetes and CVD, diabetic kidney disease, or heart failure, demonstrated a protective effect against gout flares in a secondary trial analysis and a large observational study.

Summary
The role of colchicine may expand beyond gout flare prevention to patients with existing CVD. The renal benefit of ULT among patients with gout remains unclear. SGLT-2 inhibitors may benefit diabetic patients who have gout as a comorbidity.

Keywords
cardiovascular disease, chronic kidney disease, colchicine, comorbidities, febuxostat, gout, multimorbidity, SGLT-2 inhibitors

INTRODUCTION

Multimorbidity is typically defined as the co-existence of two or more chronic conditions in an individual [1]. Its importance in the care of individuals with rheumatic diseases is evident because multimorbidity is the norm in this population [2]. Gout is strongly associated with multimorbidity—cardiovascular disease (CVD), hypertension, renal disease, diabetes, and metabolic syndrome [3]. Although the causal relationships between gout and these related morbidities remain unclear [4], physicians must effectively manage these multimorbid patients. Management options that address several co-existing chronic conditions, including gout, would be ideal. Herein, we review recent literature on pharmacological therapies that may have such potential.

Gout treatments for the management of cardiovascular risks

Anti-inflammatory therapies that have roles in the management of gout [5] saw some success in recent studies (Table 1) examining the secondary

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Curr Opin Rheumatol 2021, 33:145–154

DOI:10.1097/BOR.0000000000000784

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KEY POINTS

- Patients with gout often experience multimorbidity, thus, medications that can address both gout and comorbidities are ideal.
- Colchicine, regularly used for gout flare prophylaxis and treatment, demonstrated cardiovascular benefits among high CV risk patients with or without gout.
- Allopurinol, a cornerstone medication for chronic gout management, did not demonstrate renoprotection among patients without gout although implication for patients with gout remains to be seen.
- SGLT-2 inhibitors, a relatively new class of antidiabetics, has a uricosuric property and may protect against gout flares.

prevention of CVD in patients with or without gout [6,7,8▪▪,9▪▪]. The rationale for examining anti-inflammatory therapies' roles in CVD came from the inflammatory hypothesis of atherosclerosis [10–13]. Cholesterol crystals [14,15], like monosodium urate crystals [16], can activate the inflammasome and interleukin-1β pathway and others. As a result, studies have been conducted with canakinumab and colchicine.

Canakinumab

Canakinumab is an antiinterleukin-1β monoclonal antibody [17], which has been shown to be effective for gout flare treatment and prevention [18–21]. The 2020 American College of Rheumatology (ACR) guideline for the management of gout [5] conditionally recommends an interleukin-1β inhibitor for a gout flare when the first-line anti-inflammatory therapies (colchicine, nonsteroidal anti-inflammatory drugs, or glucocorticoids) are ineffective, not tolerated, or contraindicated.

In the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) [7], canakinumab vs. placebo were compared in patients with a history of myocardial infarction (MI) and had high-sensitivity C-reactive protein $\geq$2 mg/L despite ongoing conventional therapies. The primary endpoint of interest was a composite of cardiovascular death, nonfatal MI, and nonfatal stroke. Patients were randomized to canakinumab ($n = 6717$) and placebo ($n = 3344$). The mean age was 61 years, 25% were women, and 7.5% had a history of gout [22]. During the median 44 months of follow-up, the hazard ratio (HR) was 0.88 [95% confidence interval 0.79, 0.97], favoring canakinumab. In a follow-up study [22], a 52% reduction in gout flares was seen. Although a CVD indication was not approved for canakinumab

Table 1. Recent clinical trials on repurposing gout-relevant anti-inflammatory medications for cardiovascular outcomes

Study	Medication	Patients	CV results	Gout flare results
CANTOS [7]	Canakinumab vs placebo	Patients with previous MI ($\geq$ 30 days) and hsCRP $\geq$ 2mg/L (median 4.1 mg/L); 7.5% had gout	HR 0.88 [0.79, 0.97] for primary composite cardiovascular endpoint (median follow-up 44.4 months)	Gout flare HR 0.48 [0.36, 0.63] [22]
COLCOT [8▪▪]	Low-dose colchicine (0.5mg/day) vs placebo	Patients within 30 days of MI. Prevalent colchicine users excluded; median hsCRP 4.3 mg/L among a subset; gout prevalence unreported	HR 0.77 [0.61, 0.96] for primary composite cardiovascular endpoint (median follow-up 22 months)	Not reported
LoDoCO2 [9▪▪]	Low-dose colchicine (0.5mg/day) vs placebo	Patients with chronic coronary artery disease confirmed by imaging and stable for $\geq$ 6 months; prevalent colchicine users excluded; hsCRP unreported; 8% had gout	HR 0.69 [0.57, 0.83] for primary composite cardiovascular endpoint (median follow-up 28.6 months); Strong heterogeneity by country: Australia 0.51 [0.39, 0.67]; The Netherlands 0.92 [0.71, 1.20]	Gout flare HR 0.40 [0.28, 0.58]
COPS [33▪]	Low-dose colchicine (0.5mg/day; doubled during first month) vs placebo	Patients presented with acute coronary disease confirmed with angiography; prevalent colchicine users excluded; hsCRP unreported; gout prevalence unreported	HR 0.65 [0.38, 1.09] for primary composite cardiovascular endpoint (follow-up 12 months);	Not reported

CANTOS, Canakinumab Anti-Inflammatory Thrombosis Outcomes Study; COLCOT, Colchicine Cardiovascular Outcomes Trial; COPS, COlchicine in Patients with acute coronary Syndromes; CV, cardiovascular disease; LoDoCo2, Low-Dose Colchicine 2; HR, hazard ratio; hsCRP, high-sensitivity C-reactive protein; MI, myocardial infarction.

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partly due to cost and infection risk concerns [23,24], CANTOS marked a new era and prompted the search for more affordable alternatives [13,25,26].

Colchicine

Colchicine has been used for gout management for hundreds of years [27]. The 2020 ACR guideline [5] strongly recommends low-dose colchicine as one of the first-line therapies for the management of gout flares and for flare prophylaxis upon urate-lowering therapy (ULT) initiation for at least 3–6 months (with continuation as needed). Colchicine's anti-inflammatory activity includes inhibition of microtubular assembly, which in turn disrupts inflammasome activation, interleukin-1β release, neutrophil migration, and phagocytosis [28,29]. These sites of actions are at play in gout flare [30,31] and implicated in atherosclerotic CVD events [32]. Several trials [8▪▪,9▪▪,33▪] examined the potential CVD protective effect of colchicine in patients with or without gout [6,34,35].

In the Colchicine Cardiovascular Outcomes Trial (COLCOT) [8▪▪], the investigators examined the effect of low-dose colchicine (0.5 mg per day) vs placebo in patients who suffered MI within the last 30 days. The primary endpoint of interest was composite consisting of cardiovascular death, resuscitated cardiac arrest, MI, stroke, or coronary revascularization. Among a total of 4745 patients (enrolled mean 14 days after MI), 2366 patients were randomized to low-dose colchicine, and 2379 were randomized to placebo. The mean age was 60.6 years, and 19% were women. The proportion with gout was not reported. Nearly 100% were on aspirin, a nonaspirin antiplatelet agent, and a statin. During the median 23 months of follow-up, the colchicine arm had a 23% reduction in the hazard (HR 0.77 [0.61, 0.96]). The protective effects were particularly prominent for stroke (HR 0.26 [0.10, 0.70]) and revascularization (0.50 [0.31, 0.81]). A small, but statistically significant increase in pneumonia was observed in the colchicine group (0.9% vs. 0.4%) although no increase in cytopenia was found. A cost-effectiveness analysis demonstrated that low-dose colchicine is cost-*saving* (save money as well as quality-adjusted life years) in secondary prevention of CVD, assuming cheap generic colchicine in the Canadian healthcare system [36].

The Low-Dose Colchicine 2 (LoDoCo2) trial [9▪▪] was designed as a placebo-controlled, double-blind trial motivated by an earlier open-label (LoDoCo) trial [6]. Patients enrolled had coronary artery disease confirmed by imaging and were stable for at least 6 months. The primary endpoint of interest was a composite of cardiovascular death, MI, ischemic stroke, or coronary revascularization. Patients who tolerated colchicine during a 1-month run-in (15% dropped out) were randomized to low-dose (0.5 mg per day) colchicine ($n = 2762$) or placebo ($n = 2760$). The mean age was 66 years and 15% were women. History of gout was reported in 8% of patients, a number comparable to CANTOS [22]. Nearly 100% were on an antiplatelet agent or anticoagulant, and 97% were on a lipid-lowering agent. During the median 29 months of follow-up, colchicine reduced the hazard by 31% (HR 0.69 [0.57, 0.83]). There was a major treatment effect heterogeneity across the two countries involved in the study (HR in Australia 0.51 [0.39, 0.67]; HR in the Netherlands 0.92 [0.71, 1.20]). More noncardiovascular deaths were observed in the colchicine group (HR 1.51 [0.99, 2.31]). No increase in neutropenia was reported. Gout flare was reduced by 60% (HR 0.40 [0.28, 0.58]).

The COlchicine in Patients with acute coronary Syndromes trial [33▪] enrolled patients presented with acute coronary syndrome and imaging-confirmed coronary stenosis. The primary endpoint of interest was a composite of all-cause mortality, acute coronary syndrome, coronary revascularization, and noncardioembolic ischemic stroke. Patients were randomized to low-dose (0.5 mg per day; double dose during the first month) colchicine ($n = 396$) or placebo ($n = 399$). During the 12 months of follow-up, the HR estimate for colchicine was 0.65 [0.38, 1.09]. Although the estimate was imprecise, more all-cause deaths were in the colchicine group (8 deaths in the colchicine arm [3 CV deaths; 2 pneumonia deaths after early colchicine discontinuation; 2 likely unrelated cancer deaths; 1 presumed sepsis] vs 1 CV death in the placebo arm).

Notably, all of these colchicine trials are *secondary* prevention trials among patients who already had CVD, and nearly all were on lipid-lowering and antiplatelet therapy (Fig. 1). The importance of such conventional cardiovascular risk management before turning to anti-inflammatory therapies cannot be overemphasized. It is estimated that 14% of patients with gout have concurrent coronary artery disease [3]. There may be an additional role for colchicine in such patients for residual inflammatory cardiovascular risk [26] after sufficient conventional cardiovascular risk management. Potential signals on rare severe adverse events were not consistent across the three trials and require further investigation. However, the lack of myelosuppression in all three is reassuring.

Gout treatments for the management of chronic kidney disease risks

There is a well established connection between hyperuricemia and future incidence and progression

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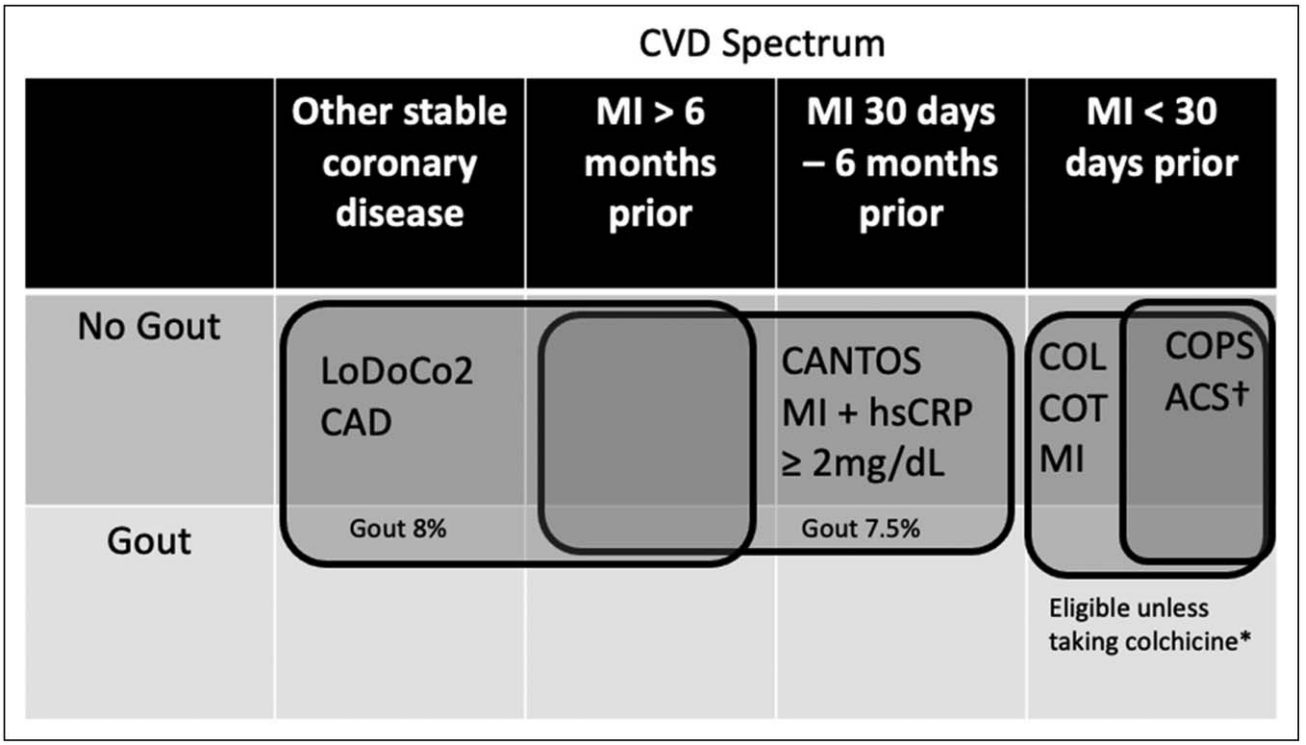

FIGURE 1. Patient characteristics represented in anti-inflammatory trials for CVD. ACS: acute coronary syndrome; CANTOS, Canakinumab Anti-Inflammatory Thrombosis Outcomes Study; COLCOT, Colchicine Cardiovascular Outcomes Trial; COPS, COlchicine in Patients with acute coronary Syndromes; CVD, cardiovascular disease; LoDoCo2, Low-Dose Colchicine 2; MI, myocardial infarction. ∗Neither COLCOT nor COPS reported % of prevalent gout although gout did not constitute an exclusion criterion by itself. †COPS included unstable angina in addition to MI. Enrollment was during the index hospitalization for ACS (more acute phase than COLCOT). These studies did not exclude patients with gout, although COLCOT and COPS did not clarify the proportion with prevalent gout. The patient population of LoDoCo2 do not overlap with the other two colchicine studies, making them complementary. Although all these studies were motivated by residual inflammatory CVD risk, only CANTOS explicitly required high hsCRP levels. A subset of patients in COLCOT had an elevated hsCRP (4.3 mg/L; similar to 4.2 mg/L in CANTOS).

of chronic kidney disease [37–41]. Earlier small-scale clinical trials suggested the potential benefit of ULT for chronic kidney disease (CKD) [42]. However, results from most recent trials are mixed (Table 2) [43▪,44▪▪,45▪▪].

Allopurinol

Allopurinol, a purine-analog xanthine oxidase inhibitor [46], is the preferred first-line urate-lowering agent in the 2020 ACR guideline [5], including for patients with gout and stages 3 and 4 CKD. Its potential role beyond gout has attracted interest; however, two trials investigating the roles of allopurinol in slowing CKD progression in those without gout gave negative results during the review period [44▪▪,45▪▪].

In the Controlled trial of slowing of Kidney Disease progression From the Inhibition of Xanthine oxidase (CKD-FIX) [44▪▪], the investigators enrolled patients with stage 3 or 4 CKD (eGFR 15–59 mL/min/1.73 m^2) and high urine albumin to creatinine ratio [47] or evidence of progression of CKD in the preceding $\leq$ 12 months (estimated glomerular filtration rate (eGFR) decrease by $>$ 3.0 mL/min/1.73 m^2) [48]. Patients with a history of gout according to the 2015 classification criteria [49] were excluded, and no specific requirement existed for serum uric acid (SUA) levels. The primary outcome measure of interest was a difference in the average annual slopes in the eGFR. They randomized $n = 182$ to allopurinol and $n = 181$ to placebo. The initial dose for allopurinol was 100 mg per day, which was escalated every four weeks up to 300 mg per day as tolerated. The mean age was 62 years, 37% were women, and the baseline SUA was 8.2 mg/dL. During the 104 weeks of follow-up, both groups experienced similar per-year declines in their eGFR: −3.33 [−4.11, −2.55] ml/min/1.73 m^2 in the allopurinol group and −3.23 [−3.98, −2.47] ml/min/1.73 m^2 in the placebo group. The annual slope difference was near zero (−0.10 [−0.18, 0.97] ml/min/1.73 m^2), whereas SUA was reduced by

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Table 2. Recent clinical trials on repurposing urate-lowering therapy for renal protection in patients *without* gout at baseline

Study	Medication	Patients	Renal results	Gout results
CKD-FIX [44▪▪]	Allopurinol vs placebo	Patients with stage 3 or 4 CKD, high albumin:creatinine ratio. Hyperuricemia was not an eligibility criterion (mean baseline SUA 8.2 mg/dL). No history of gout.	Slope difference −0.10 [−1.18, 0.97] ml/min/1.73 m^2 per year for eGFR during 104 weeks of follow-up	Gout incidence 2% in allopurinol arm vs 6% in placebo arm; SUA reduction 2.9 mg/dL
PERL [45▪▪]	Allopurinol vs placebo	Patients with type 1 diabetes (mean duration 34.6 years; A1c 8.2%) and eGFR of 40.0 to 99.9 ml/min/1.73 m^2. Hyperuricemia was not an eligibility criterion (mean baseline SUA 6.1mg/dL). No history of gout.	Mean difference 0.001 [−1.9, 1.9] mgl/min/1.73 m^2 for measured GFR after 2-mo washout	Gout results not reported; SUA reduction 2 mg/dL
FEATHER [63▪]	Febuxostat vs placebo	Patients with stage 3 CKD and asymptomatic hyperuricemia (eligibility 7.0–10.0 mg/dL; mean baseline SUA 7.8 mg/dL). No history of gout.	Slope difference 0.70 [−0.21, 1.62] mL/min/1.73 m^2 per year for eGFR	Gout incidence 0.9% in febuxostat arm vs 5.9% in placebo arm; SUA reduction 4.2 mg/dL
FREED [43▪]	Febuxostat vs usual care (open label)	Patients with asymptomatic hyperuricemia (eligibility SUA 7–9 mg/dL; mean baseline SUA 7.5 mg/dL) with hypertension, type 2 DM, renal disease, or history of cerebral or cardiovascular disease. No history of gout	HR 0.75 [0.59, 0.95] for primary composite event during 36 months of follow-up primarily due to renal impairment (HR 0.75 [0.59, 0.95])	Gout incidence 1.1% in febuxostat arm vs 2.6% in usual-care arm; SUA reduction 2.3 mg/dL

CKD, chronic kidney disease; CKD-FIX, Controlled trial of slowing of Kidney Disease progression From the Inhibition of Xanthine oxidase; (e)GFR, (estimated) glomerular filtration rate; FEATHER, Febuxostat versus placebo randomized controlled trial regarding reduced renal function in patients with hyperuricemia complicated by chronic kidney disease stage 3; FREED, Febuxostat for Cerebral and CaRdiorenovascular Events PrEvEntion StuDy; HR, hazard ratio; PERL, Preventing Early Renal Function Loss; SUA, serum uric acid.

2.9 mg/dL, indicating no clinically meaningful effect of allopurinol for halting CKD progression in this population of patients with CKD *without* gout despite their generally high SUA.

The Preventing Early Renal Function Loss (PERL) study [45▪▪] focused on the potential benefit of allopurinol in a more specific population. Patients were required to have type 1 diabetes diagnosed before age 35 for $\geq$ 8 years, have been receiving insulin since diagnosis, have albuminuria (or per-year eGFR decrease by $>$ 3.0 mL/min/1.73 m^2), have eGFR between 45 and 99.9 ml/min/1.73 m^2, and have SUA $\geq$ 4.5 mg/dL. Patients with gout and those who used any ULT within 3 months were excluded. The primary outcome measure of interest was an iohexol-based measured GFR after 3 years of treatment and 2 months of washout (to avoid transient hemodynamic effects) [50]. Patients were randomized to allopurinol ($n = 263$) or placebo ($n = 267$). The initial allopurinol dose was 100 mg per day for four weeks in the allopurinol arm. Thereafter, patients in this arm received 200, 300, or 400 mg of allopurinol, depending on their eGFR. The mean age was 51 years with a mean diabetes duration of 35 years, 34% were women, and the baseline SUA was 6.1 mg/dL. After the follow-up and washout, both groups had a mean GFR of 61.2 ml/min/1.73 m^2 with a virtually zero difference: 0.001 [−1.9, 1.9] ml/min/1.73 m^2, despite sustained SUA reduction of 2 mg/dL during treatment (only about 0.2 mg/dL after the 2-month washout). The study indicated no clinically meaningful benefit in this specific patient population with long-standing type 1 diabetes and CKD *without* gout.

As it is not ethical to randomize those with an existing indication for allopurinol, [51] both studies excluded patients with gout and did not specifically require hyperuricemia (Fig. 2). As a result, allopurinol dosing was mainly based on eGFR and toleration without a strict target SUA level. The results from these trials do not encourage the use of allopurinol in patients with CKD *without* other existing indications for allopurinol; however, the implications for patients with gout needing ULT remains unknown. A potentially important finding from these two trials is a similar increase in the all-cause mortality in the allopurinol groups compared to placebo. A pooled estimate in a recent letter [52] found a relative risk estimate for death of 2.10 [1.00, 4.42]. This may further complicate the ongoing discussion of the absolute and relative safety of urate-lowering therapies [53–57].

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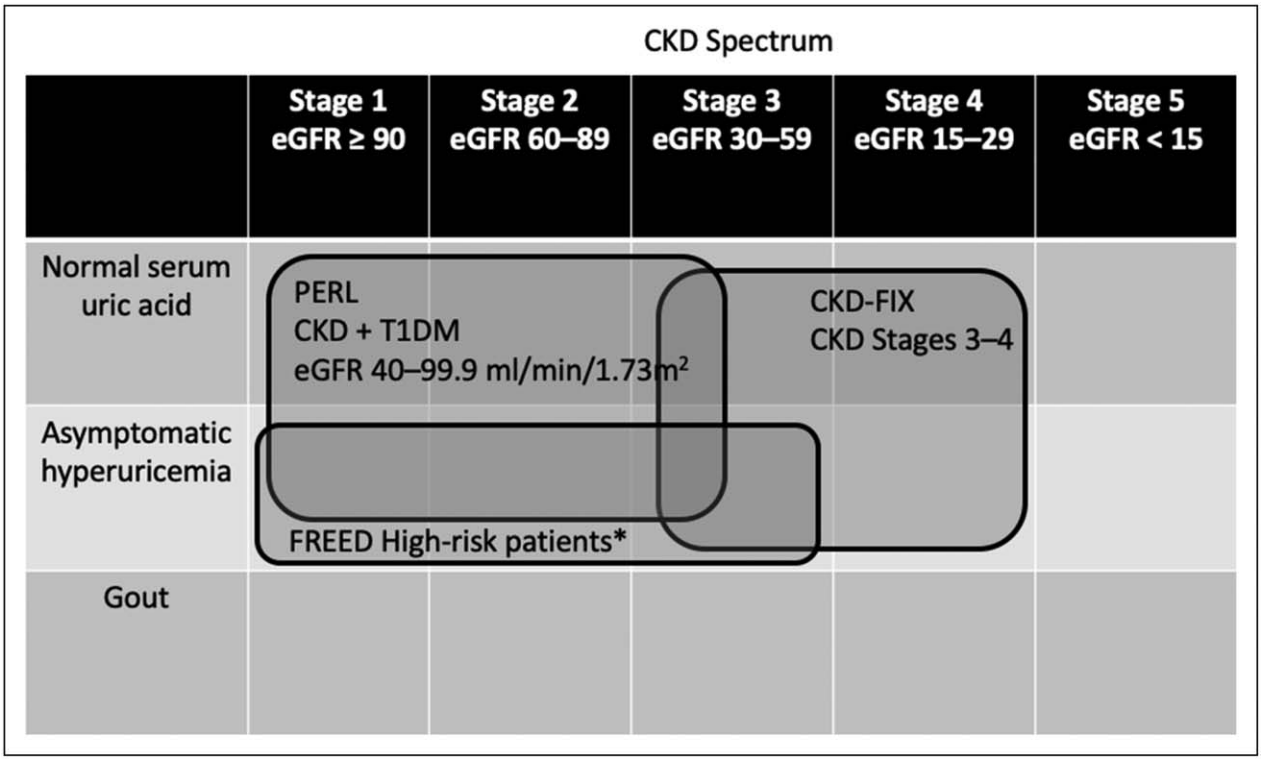

FIGURE 2. Patient characteristics represented in urate-lowering therapy trials for CKD. CKD, chronic kidney disease; CKD-FIX, Controlled trial of slowing of Kidney Disease progression From the Inhibition of Xanthine oxidase; eGFR, estimated glomerular filtration rate; FREED, Febuxostat for Cerebral and CaRdiorenovascular Events PrEvEntion StuDy; PERL, Preventing Early Renal Function Loss; T1DM, type 1 diabetes mellitus. ∗FREED enrolled patients with high risk for cerebro-cardiovascular or renal disease. Importantly, all three studies excluded patients with existing gout at baseline (i.e., those with a clear indication for urate-lowering therapy), limiting their generalizability to patients with gout. FREED explicitly targeted patients with asymptomatic hyperuricemia, while PERL and CKD-FIX included patients with normal hyperuricemia. CKD-FIX focused on patients with more advanced CKD.

Febuxostat

Febuxostat is a nonpurine xanthine oxidase inhibitor [58] with potent SUA-lowering action [59,60]. It is considered a second-line ULT in the 2020 ACR guideline for gout [5] because of its higher cost and potential safety concerns raised in a cardiovascular safety trial among patients with gout compared to allopurinol [53]. This finding resulted in a US Food and Drug Administration warning [61] although a more recent reassuring trial result [62] may prompt reconsideration. Nonetheless, there is a continued interest in febuxostat's potential benefits beyond gout.

In the Febuxostat for Cerebral and CaRdiorenovascular Events PrEvEntion StuDy (FREED), [43▪] the investigators examined the potential benefit of febuxostat in elderly (≥ 65 years) patients with hyperuricemia (SUA 7–9 mg/dL) and vascular risk factors (hypertension, type 2 diabetes, eGFR 30–60 ml/min/1.73 m^2, or cerebro-CVD). Patients with gout were excluded. The primary endpoint of interest was a composite of death, cerebral and cardiovascular events, hospitalization for heart failure, renal impairment, and new atrial fibrillation. Patients were randomized to the febuxostat group ($n = 537$) and the nonfebuxostat group ($n = 533$). The assignment was open-label. Febuxostat was initiated at 10 mg per day and doubled every 4 weeks until 40 mg per day. The mean age was 76 years, 31% were women, and the baseline SUA was 7.5 mg/dL. During the median 35 months of follow-up, the composite endpoint was reduced by 25% (HR 0.75 [0.59, 0.95]) in the febuxostat arm whereas SUA was reduced by 2.3 mg/dL. Most of the outcomes were renal impairment (development of or progression to albuminuria and proteinuria, doubling of serum creatinine, or end-stage renal disease; HR 0.75 [0.56, 0.99]). The eGFR decline was less steep in the febuxostat group (−0.37 [−2.32, 1.44] vs −0.69 [−2.63, 1.39] ml/min/1.73 m^2), but did not reach statistical significance.

Unlike the two allopurinol trials, [44▪▪,45▪▪] FREED required asymptomatic hyperuricemia as an eligibility criterion, [43▪] although patients in CKD-FIX had a higher mean SUA (7.5 vs 8.2 mg/

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Table 3. Recent studies on postulated benefit of nongout medications for gout outcomes

Study	Medication	Primary indication	Patients	Gout results
CANVAS [70▪] (Secondary analysis)	Canagliflozin (SGLT2i)	Type 2 diabetes	Patients with type 2 diabetes and elevated cardiovascular risk, eGFR $\geq$ 30ml/min/1.73m^2. Mean baseline SUA 5.85mg/dl. 5% had history of gout.	HR 0.53 [0.40, 0.71] for composite endpoint of gout flare or need for chronic gout medication
Fralick *et al.* [71▪] (Observational)	SGLT2i	Type 2 diabetes	Patients with type 2 diabetes diagnosis with indication for antidiabetics and no prevalent diagnosis of gout.	HR 0.64 [0.57, 0.72] for incident gout as defined as inpatient diagnosis of gout or outpatient diagnosis and medication use
FIELD [67▪] (Secondary analysis)	Fenofibrate	Hyperlipidemia	Patients with type 2 diabetes but no clear indication of lipid-modifying therapy. 7% had history of gout.	HR 0.54 [0.41, 0.70] for gout event during median 5 years of follow-up

CANVAS, Canagliflozin Cardiovascular Assessment Study; eGFR, estimated glomerular filtration rate; HR, hazard ratio; SGLT2i, sodium-glucose cotransporter-2 inhibitor; SUA, serum uric acid.

dL). Other differences include SUA-driven febuxostat titration, open-label design, and extremely broad composite outcome rather than GFR outcome. An earlier placebo-controlled febuxostat study with an eGFR endpoint did not find a significant difference [63▪].

Nongout medications that may benefit patients with gout

Some treatment options originally designed for nongout indications have demonstrated potential benefits in the management of patients who have gout as comorbidity, for example, losartan [64,65] fenofibrate, [66,67▪] and nonpurine-focused weight loss diet [68,69]. In this review cycle, two studies of sodium-glucose cotransporter-2 inhibitor (SGLT2i) were notable (Table 3) [70▪,71▪].

Sodium-glucose cotransporter-2 inhibitors

The SGLT2i is a relatively new class of oral antidiabetic medication, which blocks glucose reabsorption in the renal tubules and causes glycosuria [72,73]. This is beneficial not only in reducing glycemia but weight (via caloric loss) and blood pressure (via osmotic diuresis). In the current diabetes guideline, [74] SGLT2i is recommended after dietary modification, exercise, and metformin (first-line for all) in patients high risk or with atherosclerotic CVD, CKD, or heart failure [73]. Two recent studies examined SGLT2i's protective effect against gout flares, [70▪,71▪] prompted by previous findings of SGLT2i's glycosuria-induced uricosuric effects via renal tubular glucose transporter 9 [75,76].

In the Canagliflozin Cardiovascular Assessment Study (CANVAS) Program, the investigators pooled two trials, initially designed for patients with type 2 diabetes and elevated cardiovascular risks [77–79]. Patients were randomized to canagliflozin ($n = 5795$) and placebo ($n = 4347$). The original endpoint of interest was a composite of cardiovascular death, nonfatal MI, or nonfatal stroke, for which the canagliflozin group demonstrated superiority (HR 0.86 [0.75, 0.97]). In the posthoc analysis for gout, [70▪] the researchers define the outcome of interest as a composite of gout flare or gout medication (ULT, colchicine, nonsteroidal anti-inflammatory agents, or corticosteroids) initiation. In the study, 5% had a history of gout. During the mean follow-up of 3.6 years, the HR for the composite gout flare/gout-relevant mediation outcome was 0.53 [0.40, 0.71], whereas the HR for the gout flare outcome was 0.64 [0.41, 0.99].

In a recent insurance claims study, [71▪] the investigators evaluated the potential benefit of SGLT2i for reducing the risk of incident gout outcome among type 2 diabetes patients. They compared SGLT2i initiators to glucagon-like peptide-1 receptor agonist (GLP-1RA) initiators for a new diagnosis of gout. GLP-1RA was chosen as the comparator following the active comparator principle to reduce confounding by indication [80]. These medications have similar indications among type 2 diabetes patients; [74] however, GLP-1RA is considered to have no effect on hyperuricemia or gout [81]. In the propensity score-matched cohort ($n = 119{,}530$ SGLT2i initiators; $n = 119{,}530$ GLP1Ra initiators), the HR was 0.64 [0.57, 0.72], favoring SGLT2i.

CONCLUSION

Anti-inflammatory therapies that have roles in gout flare management are finding their way into secondary prevention of CVD in patients with or without gout [7,8▪▪,9▪▪]. This may be a useful development, considering the high cardiovascular comorbidities

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that patients with gout suffer [3]. It is important to remember that these trials address residual cardiovascular risk remaining after sufficient conventional risk management [26]. ULT, the cornerstone of chronic management of gout, on the other hand, has not demonstrated the slowing of GFR deterioration in CKD trials [44▪▪,45▪▪]. All of the CKD trials reviewed excluded patients with gout; thus, they do not directly inform decisions for patients with gout who require ULT. SGLT2i, an antidiabetic medication with cardiovascular benefit [82], may be relevant for patients with diabetes and gout to prevent gout flares and to reduce the risk of incident gout in patients with diabetes [83].

Acknowledgements

None.

Financial support and sponsorship

K.Y. have received research support from the Rheumatology Research Foundation K Bridge Award and NIH K23AR076453. D.H.S's work on this manuscript was supported by NIH P30AR072577.

Conflicts of interest

K.Y. has received consulting fees from OM1, Inc. and salary support from research contracts to his institution from Corrona for unrelated work. H.K.C. reports research support from Ironwood and Horizon and consulting fees from Ironwood, Selecta, Horizon, Takeda, Kowa, and Vaxart. D.H.S. receives salary support from research contracts to his institution from Abbvie, Amgen, Corrona, Genentech, and Janssen. He also receives royalties from UpToDate on unrelated work.

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- ▪▪ of outstanding interest

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COVID-19 and autoimmune diseases

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Purpose of review
The aim of this study was to evaluate the relationship between infection with SARS-CoV-2 and autoimmunity.

Recent findings
Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome (SARS) associated coronavirus 2 (SARS-CoV-2). Although most of the infected individuals are asymptomatic, a proportion of patients with COVID-19 develop severe disease with multiple organ injuries. Evidence suggests that some medications used to treat autoimmune rheumatologic diseases might have therapeutic effect in patients with severe COVID-19 infections, drawing attention to the relationship between COVID-19 and autoimmune diseases. COVID-19 shares similarities with autoimmune diseases in clinical manifestations, immune responses and pathogenic mechanisms. Robust immune reactions participate in the pathogenesis of both disease conditions. Autoantibodies as a hallmark of autoimmune diseases can also be detected in COVID-19 patients. Moreover, some patients have been reported to develop autoimmune diseases, such as Guillain–Barré syndrome or systemic lupus erythematosus, after COVID-19 infection. It is speculated that SARS-CoV-2 can disturb self-tolerance and trigger autoimmune responses through cross-reactivity with host cells. The infection risk and prognosis of COVID-19 in patients with autoimmune diseases remains controversial, but patient adherence to medication regimens to prevent autoimmune disease flares is strongly recommended.

Summary
We present a review of the association between COVID-19 and autoimmune diseases, focusing on similarities in immune responses, cross-reactivity of SARS-CoV-2, the development of autoimmune diseases in COVID-19 patients and the risk of COVID-19 infection in patients with preexisting autoimmune conditions.

Keywords
autoimmune diseases, COVID-19, cross-reactivity, molecular mimicry, SARS-CoV-2

INTRODUCTION

Since December 2019, a novel infection named coronavirus disease 2019 (COVID-19) broke out in Wuhan, China, and has been sweeping across the globe. COVID-19 was officially declared a pandemic by WHO on 11 March 2020 [1]. The disease is caused by a newly identified strain of severe acute respiratory syndrome (SARS) associated coronavirus, which was named SARS-CoV-2 after SARS-CoV that caused the epidemic of SARS in 2002 [2].

SARS-CoV-2 belongs to the coronavirus family, which are enveloped viruses with a spherical morphology and a single-stranded RNA (ssRNA) genome [3]. The spike glycoproteins (S protein) cross through the peplos of the virus and form a crown-like surface [4]. Through the receptor binding domain (RBD) located in the S1 subunit of the S protein, the virus can ligate to the host cell receptor angiotensin-converting enzyme 2 (ACE2) and invade into the cell [5–7].

In many cases, hosts infected by SARS-CoV-2 present with flu-like symptoms, such as fever, fatigue and dry cough. Headache, myalgia, sore throat, nausea and diarrhoea can also be seen in patients with COVID-19 [8,9]. Shortness of breath

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Curr Opin Rheumatol 2021, 33:155–162
DOI:10.1097/BOR.0000000000000776

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KEY POINTS

- COVID-19 infection can be complicated by involvement of multiple organ systems.
- Immune-mediated injury contributes to the manifestations and complications of COVID-19.
- Organ damage in COVID-19 is at least in part caused by perpetuated inflammatory responses, similar to autoimmune diseases.
- SARS-CoV-2 might trigger autoimmune responses through molecular mimicry.
- COVID-19 might be complicated by the development of autoantibodies and possibly de-novo autoimmune diseases.

and hypoxemia occur in severe cases. In critical cases, the disease progresses rapidly and patients can develop septic shock and multiorgan dysfunction [10]. As such, COVID-19 can be a systemic disease affecting multiple organ systems, including the skin, kidneys, respiratory system, cardiovascular system, digestive system, nervous system and haematological system [11]. The dysregulated immune response and increased pro-inflammatory cytokines induced by SARS-CoV-2 contribute to the disease pathogenesis and organ damage, which brought attention to immune-regulatory therapy in the treatment of COVID-19 [12]. Medications used to treat autoimmune diseases are widely used in critical cases of COVID-19 [13]. Further, some autoantibodies can be detected in patients with COVID-19 [14]. These observations suggest that examining pathways known to contribute to the pathogenesis of autoimmunity might provide clues to better understand and treat COVID-19.

SIMILARITIES IN IMMUNE RESPONSES BETWEEN SARS-COV-2 INFECTION AND AUTOIMMUNE DISEASES

Autoimmune diseases are characterized by the existence of autoantibodies and perpetuated inflammatory reactions due to the loss of immune tolerance and dysregulated immune system, leading to target organ damage and malfunction [15]. These immune-mediated injuries also exist in COVID-19 (Fig. 1). Infection with SARS-CoV-2 induces immune

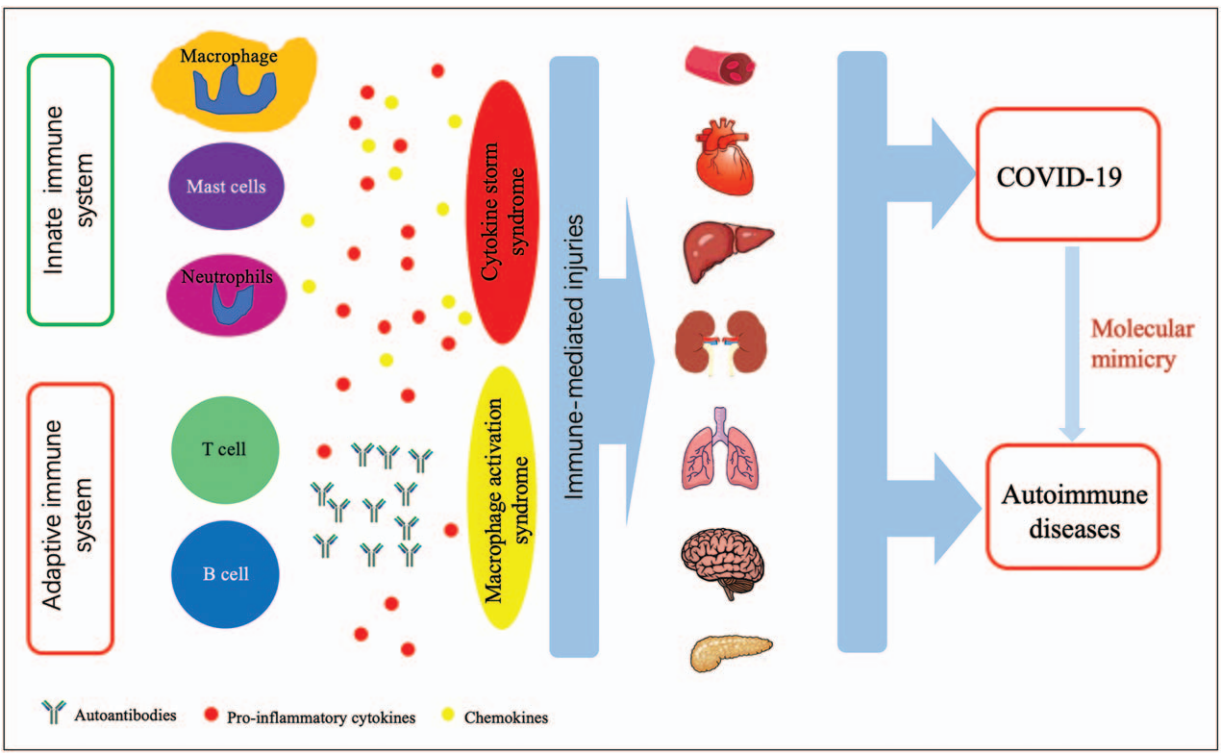

FIGURE 1. Similar immune reactions in SARS-CoV-2 infection and autoimmune diseases. Both COVID-19 and autoimmune diseases present with various clinical symptoms involving different organs and systems, such as the haematological system, cardiovascular system, digestive system, kidneys, lungs, neurological system and pancreas. Organ damage is caused by uncontrolled immune response characterized by excessive production of cytokines and overactivation of immune cells, and the break of immune tolerance leading to the production of autoantibodies. SARS-CoV-2 infection can trigger cross-reactivity through molecular mimicry, leading to autoimmunity in patients with COVID-19.

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reactions, which might have important implications in the development of vaccine strategies against this virus [16]. T cell immunity plays a central role in the control of SARS-CoV-2 infection. Antigen-specific $CD4^+$ and $CD8^+$ T cells and neutralizing antibody responses play protective roles against SARS-CoV-2, while impaired adaptive immune responses such as scarcity of naive T cells may lead to poor disease outcomes [17].

In clinical laboratory tests, lymphopenia (lymphocyte count $\leq 1.0 \times 10^9$ /l) is associated with severe illness in COVID-19 patients and might be a prognostic factor for disease severity and mortality [18–21]. Another notable haemocytological change is neutrophilia and associated excessive neutrophil extracellular traps, which paralleled lung injury in severe COVID-19 patients [12]. Therefore, the immune response is a double-edged sword in COVID-19, with outcomes affected by the degree of cytokine imbalance and activation of immune cells. Excessive production and release of pro-inflammatory cytokines and chemokines can cause severe organ damage in critical cases, which is observed in autoimmune diseases as well. In COVID-19 patients, pro-inflammatory cytokines and chemokines, including interleukin (IL)-1, IL-2, IL-6, IL-8, IL-10, IL-17, IL-18, CXCL10 and CCL2, increased significantly and the expression levels of some of these cytokines, such as IL-1, IL-6, IL-10 and IL-18, have been demonstrated to be associated with disease severity [22–25]. Similar to autoimmune diseases, damage-associated molecular patterns (DAMPs) also participate in the pathogenesis of COVID-19 and are related to disease outcome. Chen *et al.* [26] revealed that serum levels of S100A8/A9 and HMGB1 increased significantly in patients with severe COVID-19 and that significant elevation of the two DAMPs was associated with higher mortality.

Activation and infiltration of immune cells participate in the pathogenesis of organ injury in patients with COVID-19. Macrophage activation syndrome (MAS) could be a continuum of cytokine storm syndrome leading to life-threatening complications in COVID-19 [27]. In this condition, activated macrophages will produce excessive pro-inflammatory cytokines, polarize into the inflammatory M1 phenotype and exhibit cytotoxic dysfunction [28]. Recently, Conti *et al.* [29] proposed that SARS-CoV-2 activated mast cells could release histamine to increase IL-1 levels to initiate cytokine storm and aggravate lung injury. Woodruff *et al.* [30] found extrafollicular B cell activation in critically ill patients with COVID-19, similar to what has been observed in autoimmunity. Further, extrafollicular B cell activation correlated strongly with the production of high concentrations of SARS-CoV-2 specific neutralizing antibodies and poor disease outcome [30]. Peripheral blood B-cell subpopulations are altered during COVID-19. In COVID-19 patients, atypical memory B-cells ($CD21^{lo}/CD27^-/CD10^-$) expanded significantly, while classical memory B-cells ($CD21^+/CD27^+/CD10^-$) were significantly reduced [31]. Analysis of immune profiles of severe COVID-19 patients revealed an increased proportion of mature natural killer (NK) cells and decreased proportion of T-cell numbers [32].

Similar to some autoimmune and immune-mediated thromboinflammatory diseases, including lupus, antiphospholipid syndrome and ANCA-associated vasculitis, neutrophil activation and neutrophil extracellular trap production (NETosis) appear to have a pathogenic role in COVID-19. Zuo *et al.* [33▪] reported increased markers of NETs in sera from patients with COVID-19, and significantly more in patients requiring mechanical ventilation. In-vitro experiments demonstrated that sera from COVID-19 patients triggered NETosis in normal neutrophils, similar to sera from patients with antiphospholipid syndrome [33▪,34].

In severe and critical cases, immunomodulatory drugs and biological agents targeting pro-inflammatory cytokines have been applied to contain the robust immune response in COVID-19. Corticosteroids, JAK inhibitors, IL-1 blockade and IL-6 receptor antagonists, which are familiar to rheumatologists, have been used to treat COVID-19 patients [35–38]. Similarities in immunopathogenesis of COVID-19 and autoimmune diseases are summarized in Table 1.

MOLECULAR MIMICRY AND SARS-COV-2

The production of autoantibodies is a key feature of autoimmune diseases. However, the underlying mechanisms are complicated and still not fully understood. Molecular mimicry by infectious pathogens is believed to be one of the mechanisms [39]. Viral infection can disturb immunologic tolerance by exposure of antigen epitopes that elicit cross-reactive antibodies. There are a large number of reports indicating antigenic mimicry between viral and human proteins. Perhaps one of the most established examples of molecular mimicry in autoimmunity is the immune response to Epstein–Barr virus (EBV) in lupus patients [40]. An abnormal immune repose to Epstein–Barr virus Nuclear Antigen-1 (EBNA-1) can induce an autoimmune response targeting the Sm and Ro autoantigen systems [41]. Cross-reactivity between anti-EBNA-1 antibodies and myelin basic protein in patients with multiple sclerosis has also been demonstrated [42]. Moreover, EBNA-1 showed structural similarity with

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Table 1. Similarities in immunopathogenesis of COVID-19 and autoimmune diseases

Items	COVID-19 immunological features similar to autoimmune diseases	Refs.
Innate immune cells	Overactivation of monocytes, macrophages, mast cells and neutrophils. Increased proportion of mature natural killer (NK) cells.	[12,27,29,32,33▪]
Adaptive immune cells	Decreased T-cell numbers, altered B-cell subsets, dysregulation of T cells and B cells.	[17,30,31]
Cytokines and chemokines	Increased levels of IL-1, IL-2, IL-6, IL-8, IL-10, IL-17, IL-18, CXCL10, CCL2.	[22–24]
Autoantibodies	ANA, APL, lupus anticoagulant, cold agglutinins, anti-Ro/SSA antibodies, anti-Caspr2 antibody, anti GD1b antibody, anti-MOG antibody	[14,51▪,52▪,53,54▪,55–58]
Clinical conditions	Immune-mediated haemolysis, decreased white blood cell counts, cytokine storm syndrome, macrophage activation syndrome, procoagulant condition	[25,28,57,74]
Other immunopathogenesis	Increased levels of DAMPs, molecular mimicry	[26,46]

β synuclein, a brain protein implicated in multiple sclerosis, and predicted to bind HLA class II DR2b (HLA-DRB1∗15 : 01) [43]. In-silico analysis revealed that an envelope protein of human endogenous retroviruses (HERV) shares similar sequence with three myelin proteins that induced an autoimmune response in multiple sclerosis and was predicted to bind to HLA-DRB1∗15 : 01. Basavalingappa *et al.* [44] demonstrated that Coxsackievirus B3 (CVB3) infection can induce the generation of autoreactive T cells for multiple antigens.

Some epitopes from SARS-CoV-2 were revealed to exhibit cross-reactivity with autoantigens. Anand *et al.* [45] reported that a unique S1/S2 cleavage site in SARS-CoV-2 identically mimicked a FURIN-cleavable peptide on the human epithelial sodium channel α-subunit (ENaC-α), which plays a critical role in the homeostasis of airway surface liquid. Mimicry between SARS-CoV-2 and three proteins namely DAB1, AIFM and SURF1 that are present in the human brainstem pre-Bötzinger complex (preBötC) may contribute to the respiratory failure in COVID-19 [46]. In addition, SARS-CoV-2 infection can elicit autoimmune responses through molecular mimicry. Marino Gammazza *et al.* [47] compared viral proteins with human molecular chaperones and postulated that the chaperones, most of which were heat shock proteins, could participate in molecular mimicry phenomena after SARS-CoV-2 infection. Furthermore, Lucchese and Flöel [48] compared viral amino acid sequence with human autoantigens associated with immune-mediated polyneuropathies and showed that peptides embedded in immunoreactive epitopes of SARS-CoV-2 shared the same sequence with human heat shock proteins 90 and 60 that are associated with Guillain-Barré syndrome and other autoimmune diseases. Venkatakrishnan *et al.* [49▪] reported 33 distinct 8-mer or 9-mer peptides with potential cross-reactivity between SARS-CoV-2 and the human reference proteome, among which 20 human peptides have not been observed in any previous coronavirus strains. Moreover, four of these human 8-mer/9-mer peptides mimicked by SARS-CoV-2 showed similarity with host pulmonary-arterial peptides and were predicted to bind with HLA-B∗40 : 01, HLA-B∗40 : 02, and HLA-B∗35 : 01 [49▪]. A recent study analysed sharing between hexapeptides that define minimal epitopic sequences of the virus and the human proteome, and documented numerous immunoreactive epitopes shared with human proteins [50]. The results of this study imply the possibility that SARS-CoV-2 might induce cross-reactivity with host autoantigens and offer hints to possibly explain the various clinical manifestations and pathologies involving different organs and systems after SARS-CoV-2 infection.

AUTOANTIBODIES IN PATIENTS WITH COVID-19

Autoantibodies known to occur in a number of autoimmune diseases have been detected in patients with COVID-19 (Table 2). Pascolini *et al.* [14] determined the presence of antinuclear antibodies (ANA), anti-cytoplasmic neutrophil antibodies (ANCA) and anti-antiphospholipid (APL) antibodies in 33 consecutive patients with COVID-19. The results showed that 45% of the patients were positive for at least one autoantibody and patients with positive autoantibodies tended to have a worse prognosis and a significantly higher respiratory rate at admission. The positive rate for ANA was 33%, the positive rate for anticardiolipin antibodies (IgG and/or IgM) was 24% and three patients tested positive for antiβ2-glycoprotein-I

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Table 2. Autoantibodies detected in patients with COVID-19

Autoantibodies	Clinical significance	Refs.
ANA	Poor prognosis and a significant higher respiratory rate	[14]
APL	Poor prognosis and a significant higher respiratory rate Possible association with a hyperinflammatory state and thrombosis and thromboembolism	[14,52▪]
Lupus anticoagulant	A higher rate of thrombosis	[51▪]
Cold agglutinins	Haemolytic anaemia. Complicating laboratory assessment and renal replacement therapy	[55,58]
Anti-Ro/SSA antibodies	Possible association with severe pneumonia	[56]
Anti-Caspr2 antibody	Unclear	[54▪]
Anti-GD1b antibody	Unclear	[54▪]
Anti-MOG antibody	Unclear	[53]
Red cell bound antibodies	Associated with the severity of anaemia	[57]

antibodies (IgG and/or IgM) (9%). However, ANCA was negative in all patients [14]. Coagulopathy is a threatening complication of SARS-CoV-2 infection. Recently, a cohort study was performed in Montefiore Medical Center to assess lupus anticoagulant positivity in COVID-19 patients. The researchers found that patients with COVID-19 had an increased incidence of lupus anticoagulant positivity compared with controls who tested negative by COVID-19 reverse transcriptase–PCR. In addition, COVID-19 patients with positive lupus anticoagulant had an increased rate of thrombosis [51▪]. Amezcua-Guerra *et al.* [52▪] also demonstrated a higher frequency of APL antibodies in patients with severe and critical COVID-19, and that the presence of APL antibodies seems to be associated with a hyperinflammatory state with extremely high levels of ferritin, C reactive protein and IL-6, and with pulmonary thromboembolism. The data discussed above provide a possible explanation for the hypercoagulable state in severe and critical COVID-19 cases and indicate that SARS-CoV-2 can induce autoimmune responses.

In COVID-19 patients presenting with neurological symptoms, the existence of autoantibodies against contactin-associated protein 2 (anti-Caspr2), ganglioside GD1b (anti-GD1b) and myelin oligodendrocyte glycoprotein (anti-MOG) has been shown in case reports or retrospective studies [53,54▪]. However, the clinical significance of these antibodies remains unclear. In addition, there are case reports demonstrating the presence of cold agglutinins and autoantibodies against RBC antigens in critically ill patients with COVID-19 [55], and the presence of anti-Ro/SSA antibodies in patients with aggravated COVID-19 pneumonia [56]. A research including 113 samples studied red cell antibodies by direct and indirect antiglobulin test (DAT or IAT). A positive DAT was found in 46% of COVID-19 patients, which was significantly higher than that in non-COVID-19 controls. The presence of red cell membrane bound immunoglobulins contributes to haemolytic anaemia and is related to the severity of anaemia in COVID-19 [57].

DEVELOPMENT OF AUTOIMMUNE DISEASES AFTER SARS-COV-2 INFECTION

Because SARS-CoV-2 infection can break immune tolerance and trigger autoimmune responses, it is also likely to induce clinical autoimmunity. Indeed, many reports have confirmed the development of autoimmune diseases after SARS-CoV-2 infection. Cold agglutinin syndrome (CAS) and autoimmune haemolytic anaemia have been reported as a complication of COVID-19 [55,58,59]. Meanwhile, Guillain–Barré syndrome (GBS) is also emerging as an autoimmune disease that may occur in COVID-19 patients. In most cases of COVID-19 associated GBS SARS-CoV-2 antibodies cannot be detected in the cerebrospinal fluid (CSF); however, Gigli *et al.* [60] recently reported a case of GBS with a positive test for the SARS-CoV-2 antibodies in the CSF [61,62]. The mechanisms of how SARS-CoV-2 triggers GBS are debated. However, immune cross reaction between epitopes and host antigens may be a possible explanation [62]. Recently, a case of systemic lupus erythematosus has also been reported to be triggered by SARS-CoV-2 [63▪]. It is possible that additional autoimmune diseases induced by SARS-CoV-2 will be reported in the future.

RISK OF PATIENTS WITH AUTOIMMUNE DISEASES DURING THE COVID-19 PANDEMIC

Autoimmune diseases are heterogeneous and linked to a dysregulated immune system. Most of the patients with autoimmune diseases have received

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or are receiving immunomodulatory medications or biological agents. During the pandemic of COVID-19, a proportion of the autoimmune disease patients suspended their medication due to fear of the immunosuppressive effect of medications or lack of availabilities [64], and decreased medical visits because of concerns of the contagious nature of SARS-CoV-2 [65]. However, disrupted continuity of medical care and medication nonadherence are associated with rheumatologic disease flares and worsened disease activity [66]. Therefore, building a reliable telemedicine platform and education on medication adherence should be strongly recommended.

Since the beginning of this pandemic, infection risk in patients with autoimmune diseases has been a subject of interest [67▪,68,69]. The results of a cross-sectional study conducted in northeast Italy indicated that autoimmune disease patients had a similar rate of infection of SARS-CoV-2 compared with the general population [70]. Another Italian study performed in Milan also confirmed that autoimmune disease is not a risk factor of being positive for COVID-19 [71]. To the contrary, the results of a multicentre retrospective study conducted in Hubei, China, indicated that patients with autoimmune diseases might be more susceptible to SARS-CoV-2 infection compared with controls. Further, this study examined family members of the patients that resided at the same environment during the outbreak as controls [72]. Of interest, the study from Milan indicated that patients with autoimmune diseases do not have a worse prognosis compared with non-autoimmune disease individuals [71]. However, a Spanish study revealed that hospitalized patients with autoimmune diseases have a more severe course of COVID-19 [73]. At this time, until more data become available, it is crucial to emphasize the importance of physical distancing, wearing masks and frequent hand washing for everyone and especially in our patients with autoimmune diseases. Adherence to medications is also very important to prevent flares of autoimmune diseases that might result in organ damage.

CONCLUSION

COVID-19 is a novel pandemic that has had significant global health consequences. Similar to systemic autoimmune diseases, COVID-19 can present with heterogeneous and systemic clinical manifestations. To some extent, there are similarities in the immune response in both disease conditions, and organ damage in COVID-19 appears to be largely immune-mediated, similar to autoimmune diseases. The SARS-CoV-2 virus can disturb self-tolerance of host antigens at least in part through molecular mimicry. Indeed, the development of autoantibodies and sometimes organ-specific (e.g. GBS) or systemic (e.g. SLE-like disease) autoimmunity has been observed in COVID-19. Overall, more data are needed to further understand the relationship between COVID-19 and autoimmunity and characterize the risk and severity of COVID-19 in patients with preexisting autoimmune diseases.

Acknowledgements

None.

Financial support and sponsorship

This study is supported by grants from the National Natural Science Foundation for Young Scientists of China (Grant No. 81502732) to Y.L., and an urgent grant of Hunan Province for fighting against coronavirus disease- 2019 epidemic (2020SK3005) to Q.L. A.H.S is supported by the National Institutes of Health (NIH) grants number R01AI097134 and R01AR070148, and the Lupus Research Alliance.

Conflicts of interest

There are no conflicts of interest.

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Double-negative T cells in autoimmune diseases

Hao Li and George C. Tsokos

Purpose of review
TCRαβ$^+$CD4$^-$CD8$^-$ double-negative T (DNT) cells, a principal subset of mature T lymphocytes, have been closely linked with autoimmune/inflammatory conditions. However, *controversy persists regarding their ontogeny and function. Here, we present an overview on DNT cells in different autoimmune diseases to advance a deeper understanding* of the contribution of this population to disease pathogenesis.

Recent findings
DNT cells have been characterized in various chronic inflammatory diseases and they have been proposed to display pathogenic or regulatory function. The tissue location of DNT cells and the effector cytokines they produce bespeak to their active involvement in chronic inflammatory diseases.

Summary
By producing various cytokines, expanded DNT cells in inflamed tissues contribute to the pathogenesis of a variety of autoimmune inflammatory diseases. However, it is unclear whether this population represents a stable lineage consisting of different subsets similar to CD4$^+$ T helper cell subset. Better understanding of the possible heterogeneity and plasticity of DNT cells is needed to reveal interventional therapeutic opportunities.

Keywords
autoimmune diseases, double negative T cells, heterogeneity, ontogeny

INTRODUCTION

The most important hallmark of immune disorders is the activation and accumulation of T lymphocytes, the majority of which express both alpha and beta chains of the T cell receptor (TCR) and are therefore referred as αβ T cells [1]. Among αβ T cells, CD4$^+$ helper or CD8$^+$ cytotoxic T cells are most prevalent subsets [2]. However, a small population of αβ T cells which do not express both CD4 and CD8, termed 'double negative' T (DNT) cells [3,4], have been considered to contribute to the pathophysiology of a series of autoimmune diseases [4].

DNT cells were initially identified and characterized in *lpr* and *gld* mice (deficiency of either Fas or Fas ligand) in which lymphoproliferative syndrome developed due to impaired Fas-mediated apoptosis [5–9]. The massively expanded DNT cells results in the lymphadenopathy and splenomegaly, which leads to the early hypothesis that DNT cells are immunopathogenic [5]. Later on, expanded DNT cells were observed in patients with different immune disorders, including autoimmune lymphoproliferative syndrome (ALPS) [10,11], systemic lupus erythematosus (SLE) [12,13] and Sjogren's syndrome [14,15]. Although double-negative T cells only represent a small portion of αβ T cells compared with either CD4$^+$ or CD8$^+$ T cells in normal individuals [5,16], the expansion of double-negative T cells in various autoimmune diseases and the presence of DNT cells at sites of injury in different inflammatory conditions strongly suggest their critical roles in inflammation [4]. However, our understanding of DNT cell ontogeny and function still remains limited [3–5,17].

We propose that the discrepancy on the differentiation and function of DNT cells could be explained by the heterogeneity and plasticity of this type of cells.

ONTOGENY OF DOUBLE-NEGATIVE T CELLS

In healthy individuals, DNT cell only comprise a small portion of αβ T cells and are considered quiescent [5,4]. αβ T cells are derived from the developing progenitors within the thymus, the thymocytes.

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Curr Opin Rheumatol 2021, 33:163–172
DOI:10.1097/BOR.0000000000000778

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KEY POINTS

- DNT cells are expanded in various chronic inflammatory diseases and they display pathogenic or regulatory function.
- DNT cells are present in inflamed tissues and produce effector cytokines through which they exercise their function.
- It is unclear whether they represent a distinct lineage or they originate from single positive cells, whether they represent a homogenous group of cells and whether they display plasticity.

Developing thymocytes undergo a series of maturation steps before egressing from the thymus [18] and the earliest developing thymocytes lack the expression of the coreceptors $CD4^+$ and $CD8^+$ and are termed double-negative population [18,19], which leads to the hypothesis that peripheral DNT cells may represent primitive αβ T cells, which originate in the thymus but escape the late development followed by migration to the periphery (Fig. 1a) [3,20]. For late-stage thymocyte development, TCR signal strength and duration determine the lineage commitment to either $CD4^+$ or $CD8^+$ T cells. Typically, lower intensity TCR signals lead to full maturation of either $CD4^+$ or $CD8^+$ T cells, while cells with high TCR strength are deleted during the development to avoid autoimmunity [21,22]. This process has been well recapitulated by in-vitro cultured thymocytes in the presence of cortical epithelial cells [23]. Considering the fact that $CD4^+$ or $CD8^+$ expression is essential in augmenting TCR signalling by stabilizing interactions between TCR-MHC complex [24,25], it is reasonable to postulate that low or negative expression of $CD4^+$ and $CD8^+$ coreceptors protects thymocytes away from high-intensity TCR signalling mediated depletion and promotes their thymic egress [26,27]. In contrast to low concentrations of ligands that induce maturation to single positive thymocyte, double-positive thymocytes cocultured with cortical epithelial cells loaded with high concentrations of high affinity ligands acquire DNT phenotype with downregulation of both $CD4^+$ and $CD8^+$ [20,28].

However, there is sufficient evidence to suggest that DNT cells are generated in the periphery. For example, DNT cells can develop in thymectomized mice reconstituted with T cell depleted bone marrow cells [29]. The fact that mice deficient in β2-microglobulin have reduced DNT cell lymphoproliferation [30] and polyclonal DNT cells regain $CD8^+$ expression in lymphopenic environment [31], indicates that DNT may derive from peripheral mature $CD8^+$ T cells [4]. Similar evidence was generated from human studies [32,33]. First, gene expression pattern analysis revealed that DNT display more similarities with $CD8^+$ rather than $CD4^+$ T cells [32]. Second, the analysis of Vα and β usage of TCR revealed the high similarity between $CD8^+$ T and DNT in patients with ALPS [34]. The dysregulated DNT cell homeostasis in *lpr, gld* mice and ALPS patients [35–37] has directed the attention to defective apoptosis mediated by Fas dependent pathway [38,39]. The loss-of-function mutations in the Fas pathway in T cells lead to impaired apoptosis after repeated TCR engagement [9,11]. Activation-

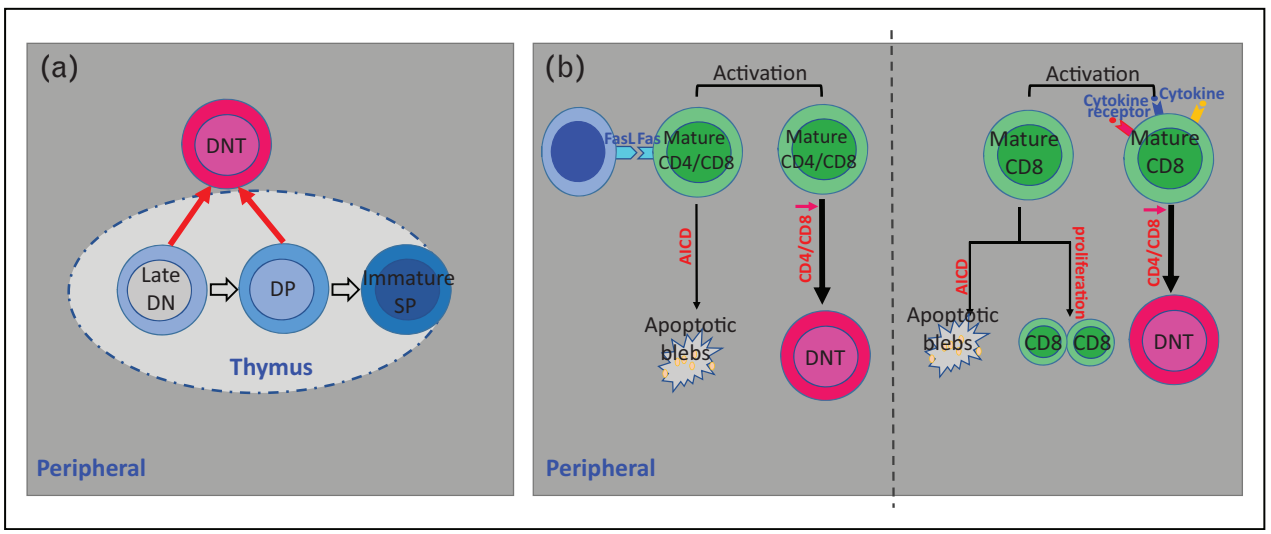

FIGURE 1. Ontogeny of double-negative T cells. (a) Peripheral DNT cells derive directly from immature DN thymocytes or from DP thymocytes through the downregulation of both $CD4^+$ and $CD8^+$. (b) Left: Activated $CD8^+$ T cells without proper apoptotic signals escape AICD and give rise to DNT cells. Right: Cytokine signal inputs help the conversion from $CD8^+$ T cells to DNT cells.

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induced cell death (*AICD*), a *Fas/FasL* dependent *negative regulator of activated T cells upon repeated TCR stimulation* [40,41], is important for the maintenance of T-cell homeostasis and abnormalities in this process may result in autoimmunity [42]. The evidence above depicts a possible model for the pathogenic DNT cell expansion in autoimmunity in which autoreactive $CD8^+$ T cells skip antigen induced AICD by losing $CD8^+$, and execute their pathogenic role *in vivo* [5,4]. Along this line, $TCR\alpha\beta^+$ CD8 T cells lost their $CD8^+$ expression upon stimulation with high concentration of anti-CD3 *in vitro* [32,33]. Adoptively transferred $CD8^+$ T cells with transgenic TCR acquired DNT-like phenotype after encountering exogenous or endogenous antigens *in vivo* [16,43,44▪▪]. Moreover, increased Ki67 expression, narrowed TCR Vβ repertoire usage and diluted T-cell receptor excision circles (TRECs) observed in DNT cells indicated the clonal proliferation and expansion possibly driven by endogenous self-antigens [44▪▪,45,46,47▪,48,49]. Of note, in-vivo antigen administration to MHC class I-restricted TCR transgenic mice on lpr background resulted in expansion of DNT cells [33], which further supported the concept that the expanded DNT cells under chronic inflammation might derive from antigen activated CD8 T cells [4,17]. However, it remains a mystery whether the absence of proper apoptotic signals or addition of supportive signals such as cytokines help activated $CD8^+$ T cells escape AICD and acquire DNT cell phenotype (Fig. 1b).

To date, the controversy on the origin of DNT cells continues. There are several possible scenarios, which are worth of attention: double-negative T cells directly originate from those immature double-negative thymocytes, which could not recognize MHC class I or MHC class II molecules but for some reason are not appropriately depleted in thymic-positive selection. Double-negative T cells represent a unique lineage, which is selected by recognition of neither class I nor MHC class II but certain unknown MHC-like molecules. There are different types of DNT cells with either intrathymic or extrathymic origin, a model which we favour most, as it fits the best for the above augments [5,17].

DOUBLE-NEGATIVE T CELLS, THE EVIL OR THE ANGEL IN INFLAMMATION

Under naive status, DNT cells represent a minor population in total αβ T cells with unrecognized roles in immune system. However, the lupus like symptoms in *lpr* or *gld* mice and disease-associated expansion of DNT cells lead to the supposition that DNT cells are assigned a pro-inflammatory role [6–9,50]. The findings that DNT cells are also expanded in patients with various inflammatory rheumatic disorders including ALPS and SLE reinforce this concept [11,12]. Evidence has emerged that supports the pathogenic role of DNT cells [4]. Ex-vivo analysis on the cytokine profiles of DNT cells from various murine models has shown the great ability to produce various inflammatory cytokines, including interleukin (IL)-2, IL-4, TNFα and IL-17A [31,43,51]. Similar results were also achieved from studies in humans with diverse autoimmune diseases [4]. In addition, DNT cells provide help to B cells to enhance autoantibody production *in vitro* [12]. Immune cell infiltration is generally considered as a major contributor of tissue damages during chronic inflammation [52]. Along this line, DNT cells are present in inflamed kidney [13,44▪▪,48,47▪], skin [53], salivary gland [14], entheses [54] and ischemic brain [55▪], which suggests they present good therapeutic targets to control inflammation in various diseases.

The activation of T cells requires signalling through TCR and the coreceptors $CD4^+$ and $CD8^+$ are essential augmenting TCR signalling [1,24,25,56]. It has been argued that the cognate TCR-antigen interaction without proper augmentation by $CD4^+$ and $CD8^+$ molecules is sufficient to drive DNT cell activation *in vivo*. Mice with concomitant deficiency of both $CD4^+$ and $CD8^+$ developed inflammatory responses and immunopathology compared with wild-type mice during acute Staphylococcal enterotoxin B infection (SEB) [57]. Of note, chronic exposure to SEB precipitated a lupus-like inflammatory disease characterized by lympho-monocytic infiltration in multiple tissues along with production of autoantibodies in these double gene deficient mice [57]. Interestingly, disease development was accompanied with the expansion of DNT cells [57]. In line with their response to SEB, in the lung of mice challenged with live vaccine strain (LSV) of Francisella tularensis, DNT cells represent the major responding T cell subset [58]. Also, in HIV-infected patients, DNT cells represent a significant portion of the cellular viral load in T cells [59,60], which suggest *in vivo* they might function similar to $CD4^+$ T cells.

In contrast to above studies, evidence has been generated suggesting that at least subsets of DNT cells exert regulatory activity [17,46]. In skin or bone marrow allograft murine model, DNT cells were capable of suppressing syngeneic $CD4^+$ or $CD8^+$ T cells in both Fas-dependent and Fas-independent manners [17]. In addition, DNT cells were also capable of inhibiting natural killer (NK) cell-mediated rejection of allogenic bone marrow through perforin-dependent killing [61]. In agreement with their role in transplantation, a number of studies

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in autoimmune diabetes revealed that transferred DNT cells can efficiently prevent diabetes onset in nonobese diabetic (NOD) mice by producing IL-10 [17,62,63]. The phenotypic counterparts of murine suppressive DNT cells have been identified also in humans [46,63]. Interestingly, in a small cohort of patients with allogeneic bone marrow transplantation, there was an inverse correlation between the frequency of circulating double-negative T cells and the severity of graft versus host diseases [64▪▪], although further mechanistic studies are needed.

HETEROGENEITY AND POSSIBLE PLASTICITY OF DOUBLE-NEGATIVE T CELLS

Variable phenotypes of double-negative T cells with diverse cytokine profiles have been reported [4,17], which indicates that double-negative T cells, similar as $CD4^+$ helper T cells [65,66], may be divided into different subsets. Five major $CD4^+$ helper T cell lineages, Th1, Th2, Th17, Tfh and Treg have been identified based on the expression of specific transcription factors and cytokine profile essential for fate determination and function [66,67]. DNT cells represent a relatively small population among total $CD3^+$ T lymphocytes with polyclonal repertoire [34,44▪▪,45], but they are selectively expanded under various inflammatory conditions. Of note, expanded DNT cells display many terminal differentiation characteristics, including Ki67 expression, a narrowed TCR Vβ repertoire and a low content of TRECs [44▪▪,46,47▪,48]. In different disease models, DNT cells exhibit completely divergent cytokine profiles. For instance, in lupus and chronic infection settings, DNT cells produce pro-inflammatory cytokine IL-17, which not only plays an essential role in the clearance of extracellular pathogens but also contributes heavily to inflammation mediated tissue damages. Moreover, in lupus-prone mice and SLE patients, DNT cells can be subgrouped based on PD1 expression [43]. Notably, $PD1^+$ but not $PD1^-$ DNT cells contain a large portion subset with self-reactive TCRs and they are the main source of IL-17 [43], which is the first solid evidence of heterogeneity among DNT cells. Similar as Th17s [68], IL-23 promotes but IL-2 attenuates IL-17 producing DNT cells [44▪▪,69,70].

In contrast, in allograft rejection and nonobese diabetes, DNT cells produce high amounts of immunosuppressive IL-10, which is essential for their regulatory capacity [17,46,62]. Successful identification of bonafide markers to separate functionally distinct DNT subsets will help reconcile the observed discrepancies. It is possible to postulate that under naive status, the regulatory DNT cells are predominant and essential for self-tolerance. During chronic inflammation, the balance of regulatory DNT cells with proinflammatory DNT cells is disturbed and pathogenic DNT cells characterized by IL-17 or other proinflammatory cytokine production become prevalent instead [67]. Although evidence suggests that DNT cells display a terminal differentiation status and proliferate poorly upon anti-CD3 stimulation [4], the possibility cannot be excluded that DNT cells are plastic. The de-novo generation of double-negative T cells from $CD8^+$ T lymphocytes [32,43,44▪▪] and the observation that DNT cells regain CD8 expression in lymphopenic environments pinpoint cell plasticity at least between DNT and $CD8^+$ T lineages [31]. Moreover, the key factors controlling the transition between different $CD4^+$ helper T subsets are various combinations of cytokines, which suppress or reinforce lineage specific transcription factors [67]. Considering the fact that reduction of TGFβ and increase of IL-23 create a milieu, which favours the expansion of IL-17 producing DNT cells [44▪▪], the cytokine environment appears to tightly control the pathogenesis of DNT cells in chronic inflammation. It is highly possible that DNT cells, similar as their $CD4^+$ counterparts, are relatively unstable and reshaped cytokine environment may result in the fate plasticity with potential ability to switch between anti and pro-inflammatory phenotypes, although more evidence is needed to support this postulate.

In addition, cell plasticity relies on cell heterogeneity. DNT cell pool might not represent a 'pure' differentiating population. Some of them might be fully differentiated with limited plasticity, whereas others may retain the flexibility because of their partial differentiation state. Exploring the key factors controlling the redifferentiation holds the promise for future treatment of DNT cell involved inflammatory diseases.

DOUBLE-NEGATIVE T CELLS IN AUTOIMMUNE DISORDERS

Autoimmune lymphoproliferative syndrome and autoimmune lymphoproliferative syndrome like diseases

ALPS is an autoimmune disorder with a progressive lymphoproliferation, massive lymphadenopathy and splenomegaly [50,71], phenotypically similar to the autoimmunity predisposed *lpr* and *gld* mice [6,8]. The massive accumulation of double-negative T cells in the blood and secondary lymphoid organs, the main manifestation of chronic nonmalignant lymphoproliferation, now is considered a key requirement for ALPS diagnosis [71–73], and this

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elevation results from a primary defect in Fas-mediated apoptosis [9,11,41,72]. In patients who develop some features of ALPS but do not fulfil the diagnostic criteria for ALPS, mutations in other components of pathways central to lymphocyte growth, activation and apoptosis have been identified including Caspase-8 and FADD [71,74]. These have been grouped into ALPS-like diseases and some patients in this category have increased DNT cells also [71,74].

Interestingly, CDR3 sequencing has revealed a significant overlap of TCR Vβ-Jβ transcripts between DNT cells and $CD8^+$ T cells from ALPS patients [34,49], which strongly suggest the at least partial $CD8^+$ origin of DNT cells in ALPS. The concept that DNT cells contribute to autoimmune symptoms in ALPS patients and autoimmunity predisposed *lpr* and *gld* mice comes from the following facts: The progressive expansion of DNT cells is closely associated with disease development [75]. The presence of autoantibodies in most ALPS patients correlates with the number of double-negative T cells [76,77]. Effective treatment ameliorates autoimmune symptoms in ALPS with significant elimination of abnormal DNT cells [78–80]. Although the elevation of DNT cells in ALPS is not in dispute, further evidence is needed to validate their pathophysiological significance.

Systemic lupus erythematosus

SLE is a clinically heterogeneous autoimmune disease with systemic inflammation and organ damage [81]. Various T cell abnormalities were reported and the expansion of DNT cells represents a prominent one [12,13,82]. The early observation that SLE patients have expanded numbers of DNT cells in the peripheral blood and this expansion correlates with disease activity leads to the supposition that expanded DNT cells contribute to the pathophysiology of SLE [13,83]. The first evidence was from in-vitro co-culture assays, which clearly demonstrated that DNT cells provide help to B cells to promote antibody production [12]. IL-17A, a pro-inflammatory cytokine, has been documented with crucial contribution for systemic inflammation and tissue damage in SLE [13,84–86]. The findings that DNT cells represent a major source of IL-17A in SLE patients reinforced the concept on DNT cell pathogenesis in SLE [4,13]. Moreover, DNT cell invasion in the kidneys of patients with lupus nephritis has also been recorded [37]. A series of experiments reported from our laboratory have demonstrated that a large portion of expanded double-negative T cells in SLE were derived from self-reactive $CD8^+$ T cells [16,31,32,44▪▪]. Self-antigens derived from apoptotic cells can activate self-reactive $CD8^+$ T cells, which give rise to double-negative T cells through the downregulation of CD8 expression on the cell surface. These cells displayed acquired proliferating or proliferated phenotype (Ki67 expression, diluted TREC and narrowed TCR repertoire) [44▪▪]. $CD8^+$ Treg cells have been described as $CD8^+$ T cells specific for antigen delivered to immune-privileged sites and to control the effector T-cell responses by $CD8^+$ and perforin dependent killing [87–89]. The whole process of conversion from $CD8^+$ T cells into DNT cells contributes to the pathogenesis of lupus based on the loss of $CD8^+$-dependent immunosuppressive potentials and the acquisition of ability to produce different pro-inflammatory cytokines and especially IL-17 [44▪▪]. In addition to TCR signalling and coreceptor signalling, cytokines provide the third signal for T cell activation and surviving [90]. The fact that skewed inflammatory cytokine environment in lupus favours the expansion of DNT cells suggests that cytokines compensate reduced TCR signalling strength due to the loss of $CD8^+$ for cell activation and survival [44▪▪].

Sjögren's syndrome, psoriasis, axial spondylarthritis and other rheumatic diseases

Sjögren's syndrome is a systemic autoimmune disease characterized by lymphocytic infiltration in salivary and tear glands [91]. Sjögren's syndrome may occur as primary disease but most often occurs in the context of other autoimmune disorders [91], including SLE and rheumatoid arthritis. Similar as in SLE, DNT cells are expanded and become the main source of IL-17 in patients with primary Sjögren's syndrome [14,15,92]. The expansion of DNT cells correlates well with disease activity and $IL\text{-}17^+$ DNT cell infiltration was detected in inflamed salivary glands [14,15].

Psoriasis is a complex inflammatory skin disease characterized by immune cell infiltration to the skin [93]. IL-17 producing DNT cell infiltration was found in the epidermis of mice with induced psoriasis [94] and patients with plaque-type psoriasis [53]. Axial spondylarthritis is another chronic inflammatory disease, which affects primarily the spine and the sacroiliac joints [95] but shares many genetic features with psoriasis [96]. Interestingly, in a widely accepted murine model of spondyloarthropathy, $IL\text{-}23R^+$ DNT cells were detected in the inflamed entheses [54]. Again, these observations reinforce the perception that DNT cells contribute heavily to pathogenesis of many inflammatory rheumatoid disorders. Furthermore, DNT cells are expanded in a subset of paediatric patients with various autoimmune diseases including mixed connective tissue

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disease (MCTD), juvenile idiopathic arthritis (JIA), juvenile dermatomyositis [97] and Behcet's disease [98], although additional investigations are required for their precise role in these patients.

Type 1 diabetes

Type 1 diabetes (T1D) is an organ-specific autoimmune disease with severe loss of pancreatic β cells [99]. Both $CD4^+$ and $CD8^+$ T cells play distinct and highly pathogenic roles in β cell destruction [100]. In contrast to the pathogenesis of DNT cells in inflammatory rheumatoid disorders listed above, a number of studies have demonstrated the immunosuppressive ability of DNT cells and their ability to inhibit the development of autoimmune diabetes [3,17]. First, a progressive loss of DNT cells with age was observed in nonobese diabetic (NOD) mice [62]. Second, adoptive transfer of DNT cells efficiently inhibited the development of autoimmune diabetes in several different diabetic mouse models [63,101,102]. Third, transfer of NOD $CD8^+$ T cells resulted in diabetes but co-transfer of NOD $CD8^+$ T cells with DNT cells did not, which indicates that DNT cells act directly on pathogenic T cells to exercise their immunosuppressive function [17,62]. However, controversies remain on the nature of immunosuppression of DNT cells. Distinct mechanisms with different molecules involved have been proposed for DNT cell mediated suppression including elimination of effector T cells by either Fas/FasL-mediated apoptosis [103,104] or perforin mediated killing [46,102,105,106] and modulation on antigen presenting cells by producing IL-10 [62,63] or IFNγ [46,107,108]. Of note, both IL-10 and IFNγ function as a double-edged sword in autoimmune diseases [109,110] and the immune environments determine whether they are beneficial or detrimental. Therefore, more mechanistic studies are needed.

THERAPEUTIC INTERVENTIONS TARGETING DOUBLE-NEGATIVE T CELLS

In autoimmune diseases wherein expanded DNT cells display distinct pathogenic capacity their selective ablation or specific modulation of the processes that render them less pathogenic should be considered for therapeutic purposes. More attention should be given to the design of specific drugs able to limit the expansion pathogenic DNT cells or if possible favour regulatory DNT activation. In light of understanding of DNT cell generation in lupus, more and more approaches directly or indirectly targeting DNT cells have been tested. In lupus-prone mice and SLE patients, a large portion of DNT cells were derived from antigen-stimulated $CD8^+$ T cells. The activation-induced chromatin remodelling and epigenetic silencing on various promoters and enhancers of *Cd8* locus might be responsible for the de-novo generation of DNT cells from $CD8^+$ T cells. As expected, the methylome of DNT cells affirmed hypermethylation on regulatory elements of *Cd8* locus [111]. In brief, the transcription factor cAMP responsive element modulator (CREM)α orchestrates DNA methyltransferase (DNMT)3a and histone methyltransferase G9a to directly enhance DNA and histone methylation on *Cd8* locus [112,113], which results in stable epigenetic silencing [113,114]. Accordingly, genetic deficiency of *Crem* in lupus-prone mice significantly ameliorates disease manifestations by reducing IL-17^+ DNT cells [115]. *DNA methylation* patterns in SLE T cells are complex with both hypomethylated and hypermethylated cytosine-guanine sites [116,117]. Generalized DNA hypomethylation in $CD4^+$ T cells has been well linked to the disease manifestation [118,119]. Surprisingly, in contrast to systemic delivery of 5-azacytidine [120], a DNA methyltransferase inhibitor, which profoundly augments disease progression [121], its targeted delivery to $CD8^+$ T cells using a nanolipogel delivery system significantly ameliorated disease severity in lupus prone mice by restraining the expansion of pathogenic DNT cells [122]. This result is consistent with the proposition that $CD8^+$ T cells acquire proinflammatory DNT cell phenotype through enhanced DNA methylation mediated $CD8^+$ loss [4]. In line with these observations, well controlled $CD8^+$ expression on $CD8^+$ T cells and DNT cells by proper modulation of epigenetic modification on *Cd8* locus should present a valuable therapeutic strategy for the treatment of autoimmune disease with involvement of DNT cells (Fig. 2a).

The expanded DNT cells in human and mice with defective Fas-mediated pathway depicted another picture, in which double-negative T cells were derived from mature T cells with failed apoptosis [5]. Along this line, DNT cells with resistance to AICD could be generated *in vitro* from Fas-sufficient T-cells with repeated anti-CD3 stimulation [32,33]. However, further studies are warranted to validate whether addition of FasL or other apoptosis inducing molecules could modulate the generation of DNT cells as expected *in vitro* and *in vivo* (Fig. 2b), as the controversies persist on the therapeutic values of FasL in autoimmune disease [123]. Fas and FasL play essential immunosuppressive roles in controlling T cell homeostasis, as recorded with the development of autoimmunity in *lpr* or *gld* mice [5]. Paradoxically, Fas also plays a proinflammatory role in certain settings, as *lpr* or *gld* mice are resistant to

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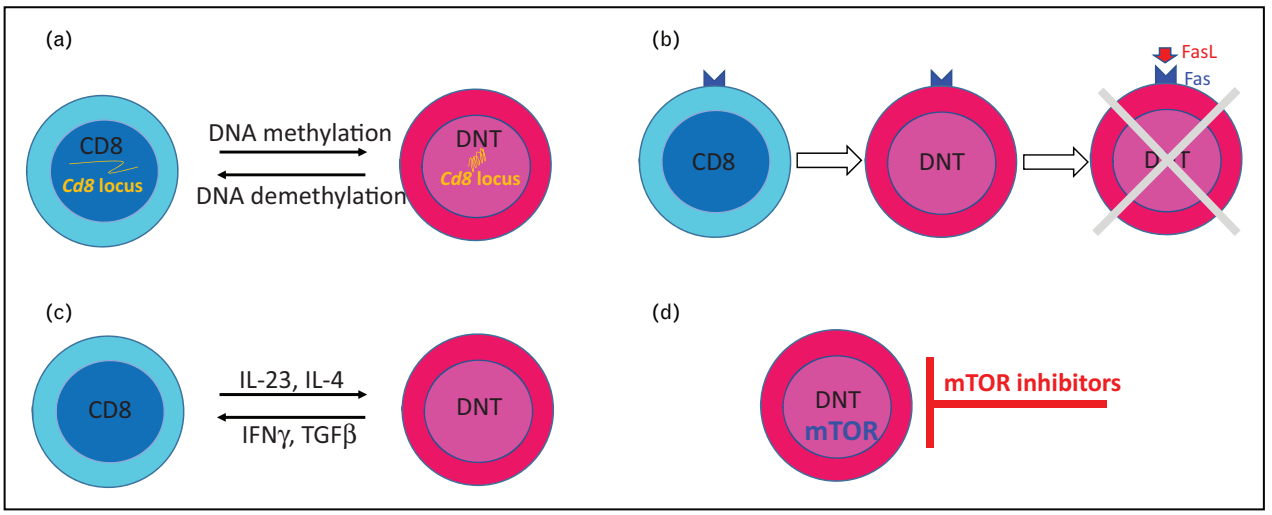

FIGURE 2. Therapeutic interventions targeting DNT cells. (a) Regulate the conversion between DNT cells and CD8$^+$ T cells through epigenetic modulation. (b) Eliminate DNT cells by adding missing signals for apoptosis. (c) Regulate the conversion between DNT cells and CD8$^+$ T cells by reshaping the cytokine milieu. (d) Inhibit DNT cell activation and expansion by targeting DNT cell metabolism.

induced rheumatoid arthritis [124] and type I diabetes [125]. The constitutive expression of Fas in many types of cells may explain the observed complexity of Fas-mediated immune response [126]. Therefore, further insights into Fas-dependent and Fas-independent DNT cell homeostasis are needed for better therapeutic strategies.

The requirements for signal 3 provided by cytokines to DNT cell activation and differentiation link the cytokine milieu to loss of CD8 expression in CD8$^+$ T cells [44▪▪,90]. It has been reported that IL-4 induced STAT6 orchestrates GATA3 for transcriptional repression of *Cd8* [114]. Interestingly, IFN-γ partially recovered CD8 expression in a subset of double-negative T cells [114], which is consistent with the observation that DNT cells could reattain CD8 expression in the proper cytokine milieu in lymphopenic hosts [31]. Furthermore, *in vivo*, elevated IL-23 along with reduced TGFβ facilitate self-reactive double-negative T-cell activation, expansion, and survival [44▪▪]. Targeting cytokines, specific intracellular kinases or transcription factors provides an alternative therapeutic choice (Fig. 2c), although caution has to be applied because of shared components between different pathways.

It has become clear that metabolic processes control the fate decision of T cell differentiation and further the function of T cells. In autoimmune diseases, the disturbed or skewed metabolic pathways in T cells have been frequently reported [127,128]. However, most studies focus on CD4$^+$ T cells and very little attention has been given to DNT cells. Observation made in a clinical trial of sirolimus in patients with active SLE showed dramatic reduction of IL-4$^+$ and IL-17$^+$ producing DNT cells 12 months after treatment [129▪▪], which strongly suggests that mTOR blockade corrects pro-inflammatory DNT cell differentiation and activation. Consistently, PP2A, a serine/threonine phosphatase, plays a key role in restraining the activation of the metabolic checkpoint kinase mTOR and the PP2A activating molecule FTY720 induced DNT cell apoptosis in lupus prone murine [130]. Thus, the development of novel therapies to control the activity of metabolic enzymes in DNT cells represents a promising exercise for treatment of autoimmune diseases (Fig. 2d).

CONCLUSION

DNT cells represent important component of the immune system [5]. Although the possibility cannot be excluded that some DNT cells are direct thymic escapes, a great portion of DNT cells are generated from peripheral CD8$^+$ T cells, which lose CD8$^+$ expression on cell surface following the stimulation with combination of various signals including TCR engagement and cytokine stimulation [17,32,43,44▪▪]. Distinct epigenetic processes are responsible for this process and more studies are wanted for more details [4]. The fact that DNT cells infiltrate various inflamed organs including the skin and the kidney in different diseases [4] along with their ability to help B cells to produce autoantibody [12] and various pro-inflammatory cytokines including IL-17 [13] underwrites their important contribution to the pathogenesis of autoimmune diseases. It is highly possible that a subset of DNT cells may instead have regulatory capacity in certain disease settings like organ transplantation and

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nonobese diabetes [17]. A growing understanding of DNT cell origin and functional features has prompted the consideration of therapeutic approaches including targeted reopening of the CD8 locus, precise modulation of cell activation and survival, inhibition of proinflammatory metabolic pathways and blockade of the inflammatory milieu which enables their generation or enabling their demise.

A number of questions needs urgent attention. A clear characterization of DNT subsets is needed through novel spectral cytometry and single cell sequencing technologies. The factors which enable the expansion of proinflammatory or regulatory DNT cells in various diseases need to be defined. Using advanced protocols, including Slide-Seq [131], the exact interaction between DNT cells and other immune cells or tissue resident cells should be defined. Prospective clinical studies are needed to define their appearance during the evolution of the disease process. Such studies may reveal that certain characteristic of DNT cells in the periphery can serve as biomarkers of organ inflammation and disease activity.

Acknowledgements

None.

Financial support and sponsorship

This worked was supported by grants RO1 AI085567 to G.C.T. and T32 DK007199 to H.L.

Conflicts of interest

There are no conflicts of interest.

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Immunopathogenesis of skin injury in systemic lupus erythematosus

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Purpose of review
Skin injury is the most common clinical manifestation of SLE and is disfiguring, difficult to treat, and incompletely understood. We provide an overview of recently published articles covering the immunopathogenesis of skin injury in SLE

Recent findings
Skin of SLE has an inherent susceptibility to apoptosis, the cause of which may be multifactorial. Chronic IFN overexpression leads to barrier disruption, infiltration of inflammatory cells, cytokine production, and release of autoantigens and autoantibody production that result in skin injury. Ultraviolet light is the most important CLE trigger and amplifies this process leading to skin inflammation and potentially systemic disease flares.

Summary
The pathogenesis of skin injury in CLE is complex but recent studies highlight the importance of mechanisms driving dysregulated epidermal cell death likely influenced by genetic risk factors, environmental triggers (UV light), and cytotoxic cells and cellular signaling.

Keywords
apoptosis, cutaneous lupus, interferon, photosensitivity, systemic lupus

INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex multiorgan autoimmune disease that results in tissue inflammation and damage. Skin inflammation, referred to as cutaneous lupus erythematosus or CLE, is the most common organ to be involved, and occurs in up to 93% of patients with SLE [1]. CLE is the first sign of systemic disease in up to 25% of cases [1]. Skin lesions can be divided into clinical subtypes according to the Düsseldorf criteria based on acuity of inflammation (acute CLE, subacute CLE, chronic CLE) or by histologic appearance of biopsy specimens. Histologically, CLE is characterized by a lichenoid pattern (interface dermatitis) with superficial and deep dermal lymphocytic infiltrate and scattered apoptotic keratinocytes. Immune complex deposition, termed the 'lupus band', along the dermoepidermal junction [2] is also found in CLE lesions and can also be detected more in sun-exposed than in sun-unexposed skin of SLE patients [3]. Recent findings in CLE have uncovered important cellular and molecular mechanisms driving skin inflammation and dysregulated epidermal cell death and highlight a need for further investigation. In this review, we will discuss our current understanding of genetic and environmental risk factors and role of cytotoxic cells and cellular signaling in the development of skin injury in SLE.

PATHOGENESIS OF SKIN INJURY IN SYSTEMIC LUPUS ERYTHEMATOSUS

Genetic risk factors

Similar to SLE, many identified genetic risk factors for CLE contribute to the function of the immune system, which has expanded our understanding of the pathogenesis of skin injury. CLE has been associated with a number of genes and gene products linked to various cellular and molecular pathways, including signaling and adhesive interactions, uptake of complement-coated particles and pathogens, defective clearance of immune complexes, and

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Curr Opin Rheumatol 2021, 33:173–180
DOI:10.1097/BOR.0000000000000770

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KEY POINTS

- Cutaneous lupus erythematosus (CLE) is the most common manifestation of SLE and is characterized by immune-mediated skin injury.
- Mechanisms of skin injury in CLE are multifactorial but involve chronic IFN overexpression, infiltration of inflammatory cells, cytokine production, and autoantibody production.
- Exacerbated epidermal cell death and inflammation prime SLE/CLE skin for inflammatory responses.
- UV light is the most important CLE trigger and amplifies epidermal injury potentially leading to systemic disease flares.

DNA degradation, which all serve to promote the initiation of autoimmunity [4]. Specific alleles in genes associated with skin injury include *ITGAM* (also known as CD11b), which is associated with an increased risk of discoid lupus erythematosus [5], and *FCGR2A* (encoding low-affinity IgG Fc region receptor IIa), which is associated with increased susceptibility to a malar rash [5]. Mutations in *TREX1* (encoding 3′ repair exonuclease 1) have been linked to chilblain lupus and are thought to increase interferon production secondary to increased nucleic acid sensing [6]. Polymorphisms in the *IFNK* gene that encodes for IFNκ, a type-I IFN, may also confer susceptibility to CLE [7]. In addition to traditional genetic variation, differences in expression of noncoding RNAs may also impact disease but further validation is required [8▪].

Environmental risk factors

Ultraviolet light

Sensitivity to sunlight and ultraviolet light (UV) is a characteristic feature of lupus erythematosus, with a reported frequency of 60–80% [9,10]. Photosensitivity is well known to be the most important environmental trigger for skin injury [11] and potentially systemic disease flares [12,13]. UV light directly induces production of chemokines and cytokines, indirectly causes keratinocyte apoptosis and necrosis, and causes displacement of nucleoproteins to the cell surface. These pathways all likely contribute to skin injury and inflammation in SLE and CLE through increasing recruitment of immune cells, production of inflammatory signals, and increasing autoantigen production and antibody binding to keratinocytes.

UV light directly increases cytokine production as it triggers keratinocytes and immune cells to release IFN, TNFα, transforming growth factor (TGF)-β, IL-1α/β, IL-6, IL-8, IL-10, and IL-17 [14–17]. These cytokines contribute to skin injury by increasing release of additional inflammatory cytokines and by recruiting inflammatory cells, both of which lead to local tissue inflammation and injury. UV irradiation increases expression of chemokine (CXC motif) ligand CXCL 9, CXCL10, and CXCL11 and (C-C motif) CCL27, which are the most highly expressed chemokines in CLE [18]. In addition, even in healthy skin, UV light induces upregulation of nucleoproteins that serve as autoantigens in SLE patients, which suggests that chronic or intense UV exposure can increase the propensity for autoantigen exposure [19▪].

UV irradiation causes DNA damage and apoptosis of keratinocytes. Nonlesional keratinocytes from lupus patients have been shown to have increased cell death following UVB irradiation compared with control suggesting a predisposition to skin injury following UV exposure [20]. Chronic type I IFN exposure in lupus skin promotes increased apoptosis of keratinocytes after UVB stimulation and blockade of IFN signaling reverses heightened cell death in SLE keratinocytes [20]. Loss of EGFR signaling is also a feature of CLE lesions [21▪], which contributes to heightened keratinocyte apoptotic response to UVB in SLE patients [22] (See Fig. 1).

Lupus-prone murine models have also demonstrated differences in UV responses compared with control. These models suggest a role for enhanced nucleic acid-triggered IFN induction in the skin as a mechanism. Rapid induction of type I IFN responses in the skin occur after UVB exposure [23▪▪]. Lupus prone *Trex1*−/− mice or MRL/*lpr* mice develop CLE-like lesions following direct injection of UV-modified DNA [24]. MRL/*lpr* mice have increased susceptibility to UV-mediated DNA release compared with wild-type [25]. Mice with an extra copy of TLR7 (BXSB males) develop lethal lupus nephritis following high dose UVB exposure; however, this does not induce a flare in female littermates or in NZB/NZWF1 mice [25]. The molecular and cellular signaling underlying these differences remains poorly understood, intriguingly, cutaneous UV exposure increases renal expression of IFN-regulated genes [23▪▪], suggesting IFNs may be a link between cutaneous and systemic inflammation.

One proposed mechanism of how UV-induced skin injury may lead to systemic inflammation is through the increased autoantigen production in the epidermis. Recent transcriptional data support upregulation of key autoantigens after UV exposure including Ro52 [19▪] and IF116, Sm, RNP, Ku, and ribosomal-P [25–27]. It is proposed that antigen upregulation coupled with increased keratinocyte

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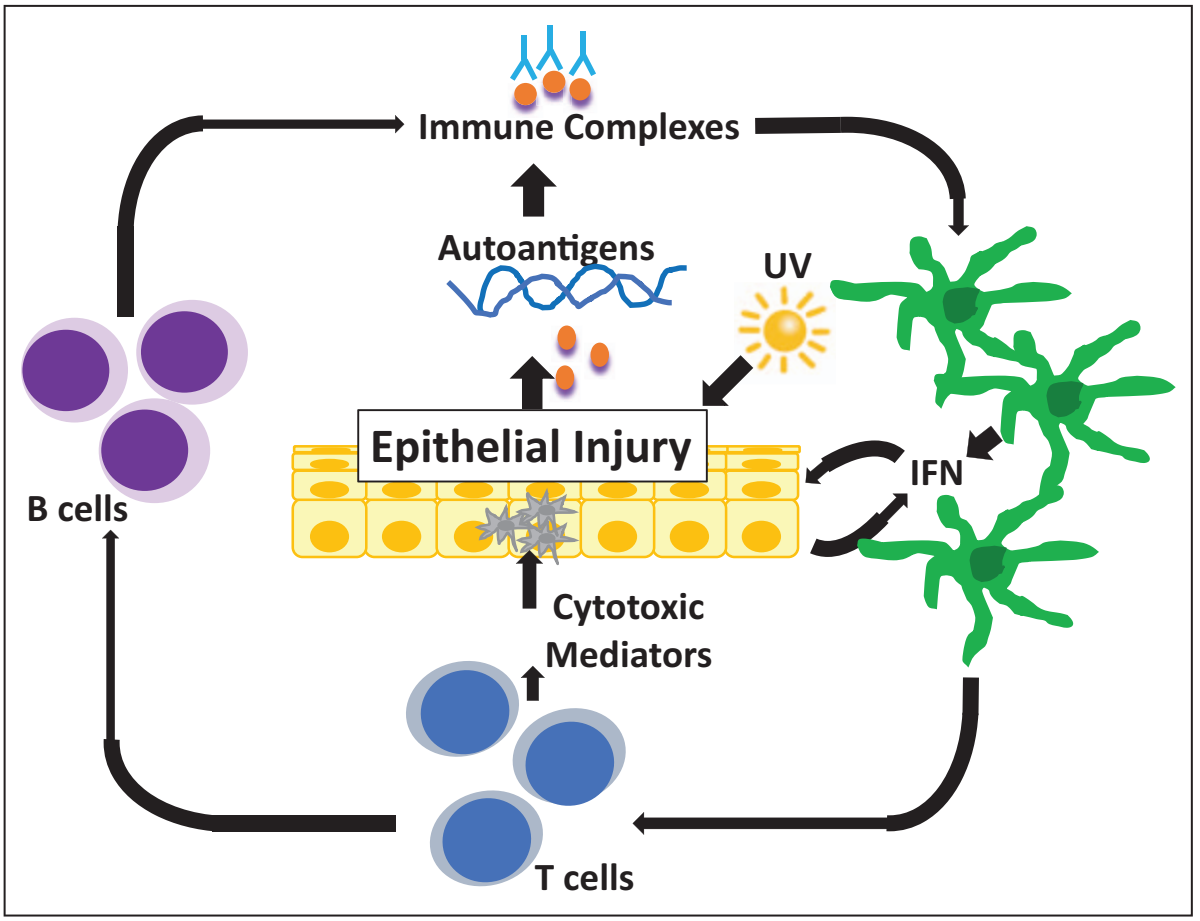

FIGURE 1. Inflammatory loop of skin injury and response in systemic lupus erythematosus. IgG and immune complex deposition are found in both lesional and nonlesional skin of patients with SLE. Additional triggers (such as UV exposure or smoking) are required for inflammation and rash to develop. Chronically elevated levels of type I IFN, specifically IFNκ, are required for the exaggerated UV-induced keratinocyte apoptosis and drive the pro-inflammatory loop by stimulating resident dendritic cells and keratinocytes to release signals to stimulate T-cell activation. The inflammatory loop is completed by T-cell activation of B cells to further produce autoantibodies potentially leading to spread of the rash and to systemic disease flare. IFN, interferon; SLE, systemic lupus erythematosus; UV, ultraviolet.

death, impaired clearance of debris [28,29] and enhanced migration of APCs to draining lymph nodes result in activation of the adaptive immune response and induction of autoantibody formation. In addition, UV irradiation results in translocation of Ro/SSA and La/SSB from the nucleus to the cytoplasmic membrane making them susceptible to binding by circulating antibodies [30–32].

Smoking

Smoking is well known to increase CLE disease activity by causing skin damage. A prospective longitudinal cohort study found that current smokers with lupus had worse disease, (demonstrated by elevated CLASI score), worse quality of life (measured by Skindex), and more often required higher levels or combination of therapies compared with nonsmokers [33,34]. Prior studies have also shown that current smokers are more likely to have refractory disease than nonsmokers [35] and that antimalarial agents may be less effective in patients with CLE who smoke [36–38]. The pathophysiology is not entirely clear, but tobacco smoke has been shown to increase inflammatory cytokines, free radicals and activate neutrophils to form neutrophil extracellular traps (NETs), further increasing cellular stress and apoptosis all of which are likely contribute to cellular injury [39].

CELLULAR CONTRIBUTORS TO TISSUE INJURY

Lymphocytes

CLE is histologically characterized by subepithelial inflammatory infiltrate of lymphocytes and

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plasmacytoid dendritic cells (pDCs). The lymphocytic infiltrate consists of subpopulation of granzyme B^+ T cells, which are considered direct markers of cytotoxic damage and central to keratinocyte death [40–42]. Granzyme B (GrB) is a serine protease that primes for apoptosis through caspase activation and cleavage of substrates that initiate DNA fragmentation [43]. Cytotoxic T-cell ligands/receptors, such as CXCL10/CXCR3 are highly expressed in lupus skin [44] and also upregulated on Th1 cells following stimulation with IFNγ [44], which is noted to present in discoid lesion more so than other CLE subtypes [21▪]. Type I IFNs also influence this process as GrB expression in lymphocytes is induced following IFNα stimulation by pDCs [45]. In addition, there is a strong correlation between epidermal and dermal IFN-regulated gene expression and the number of lesional GrB-positive lymphocytes [46]. Type I IFNs stimulate development of cytotoxic lymphocytes and could make keratinocytes more prone cytotoxic attack [47]. Although cytotoxic T-cell autoreactivity to keratinocyte-expressed proteins may drive skin injury in cutaneous LE lesions, the role of other T-lymphocyte subtypes is less clear.

INNATE IMMUNE CELLS

Innate immune cells, including pDCs and neutrophils, contribute to tissue damage by releasing inflammatory mediators and by failing to effectively clear cellular debris.

pDCs are a specialized population of bone marrow-derived cells that synthesize large amounts of type I IFN, IL-6, and TNFα in response to ligands that engage TLR7, TLR9, and immune complexes [48,49]. pDCs are rare in normal skin but are abundant in CLE lesions [50–52]. pDCs may contribute to cellular injury following UV exposure as UV light increases infiltration of pDCS in exposed skin of lupus patients vs. healthy controls [53]. pDCs have been shown to be critically involved in the pathogenesis of lupus erythematosus as depletion of pDCs in mice before disease initiation ameliorated autoimmunity [51]. Additionally, a recent phase I study utilizing an antibody against BDCA2, a pDC-specific receptor, has shown promise in improving CLE disease activity [54]. Further study of contributions of pDCs to SLE and CLE is needed.

Neutrophils may also contribute to tissue damage in CLE. As in many inflammatory states, including oxidative stress, infections or autoimmunity, neutrophils undergo a mechanism of cell death called neutrophil extracellular traps or 'NETosis' in which they extrude nuclear material [55▪▪]. NETs have been detected in the skin of patients with lupus [55▪▪] and likely contribute to immune activation by forming of immune complexes and stimulation of IFNα by pDCs [55▪▪] leading to tissue damage and accumulation of cellular debris. Intriguingly, neutrophils are increased in nonlesional skin prior to lesion onset in a murine CLE model [56]. Studies have shown that activated phagocytic cells, including monocytes and macrophages, are increased in CLE lesions [57▪▪] and importantly have an impaired ability to clear apoptotic debris [58]. Accumulation of cellular debris following cell death results in an increased propensity for autoantigen exposure and risk of production of circulating autoantibodies.

MEDIATORS OF TISSUE DAMAGE

Fas and FasL

Mande *et al.* recently developed an inducible model of murine autoimmunity that presents with skin lesions similar to CLE. In this model, transfer of T cells specific for antigen expressed by MHC II-positive cells resulted in FasL-dependent killing of keratinocytes and CLE lesion induction. Interestingly, this model required tissue damage via gamma irradiation in order for CLE lesions to occur [59]. Activated or damaged keratinocytes express T-cell chemokines, such as CXCL9, CXCL10, and CXCL11. Keratinocytes also produce IFN kappa (IFNκ), which may contribute to the strong Th1 phenotype and infiltration of immune cells. FasL was also shown to be upregulated in human CLE biopsies [59]. Further, in the Mande *et al.* model, blockade of the type I IFN receptor prevented development of skin disease and the accumulation of dermal pDCs, suggesting an IFN-dependent feedback loop in the maintenance of skin injury, even when the phenotype is driven primarily by T cells.

Tumor necrosis factor α

Tumor necrosis factor (TNF) α is increased in both the skin and serum of patients with CLE [60,61] and in the skin is upregulated by UVB exposure, in part by IL-1α signaling [62,63]. TNF increases inflammation and skin injury by producing cytokines, chemokines and adhesion molecules, such as IL-1, IL-6, CXCL8, CCL20, selectins, and ICAM-1, which increase leukocytes into lesional skin. In SCLE, an increase in the autoantigen Ro52, which is associated with photosensitivity, is increased following TNFα treatment [64]. Paradoxically, TNF α antagonists have been reported to cause lupus-like skin disease [65]. Thus, further research

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is needed regarding this pathway's role in the pathogenesis of CLE.

Tumor necrosis factor-like weak inducer of apoptosis

Another TNF-family cytokine more recently recognized to be key in propagating skin injury in SLE is TNF-like weak inducer of apoptosis (TWEAK) [66,67]. TWEAK functions as a soluble cytokine that signals through its sole receptor, fibroblast growth factor-inducible 14 (Fn14) [68]. Mechanistic studies have elucidated a role in cell proliferation, cell death, inflammation, and tissue repair [69,70]. Notably, the expression of TWEAK/Fn14 pathway is increased in the setting of tissue injury and disease [71] and may contribute to skin injury in lupus via cell death, promotion of inflammatory cytokines, and UV sensitivity. A recent study showed that keratinocytes stimulated with TWEAK produce RANTES, in an Fn14-dependent manner and also demonstrate NFκB-dependent enhanced apoptosis [72]. In *vivo*, MRL/*lpr* Fn14KO mice were shown to have significantly attenuated skin lesions compared with the FN14 wildtype mice that displayed epidermal thickening and interface dermatitis similar to CLE [72]. Further, MRL/lpr FN14KO mice develop less severe skin lesions with reduced inflammatory infiltrates following UVB treatment compared with FN14 wildtype mice [72]. The role of the TWEAK/Fn14 pathway in skin injury of primary human keratinocytes and in patients with CLE needs further investigation.

Type I interferons

Type I IFNs are central to the development of CLE and contribute to tissue injury by upregulating cytokines, chemokines, adhesion molecules as well as recruiting other inflammatory cells leading to a proinflammatory loop [53,73]. Type I IFNs are expressed in the epidermis and dermis of skin lesions, with IFN α10 and IFNκ being significantly overexpressed [20]. Sources of IFNs are likely keratinocytes (for IFNκ) and pDCs, which produce type I IFN in response to immune complexes [74]. Additionally, type I IFNs upregulate cytotoxic proteins, such as perforin and granzyme B via IFNγ in T cells, the apoptosis receptor cluster of differentiation 96 (CD95) and TNF-related apoptosis-inducing ligand (TRAIL) [45,75,76]. TRAIL is a keratinocyte-produced IFN-dependent chemokine that is upregulated in CLE skin [76]. TRAIL's proapoptotic receptor is also found on keratinocytes in CLE lesions and is upregulated upon stimulation with IFNα [76]. UV light, the main trigger of keratinocyte apoptosis and skin injury, also overstimulates pDCs to produce IFNs in SLE compared with controls [77]. Chronic overexpression of IFNκ promotes this process, as it is required for UVB-induced apoptosis of keratinocytes [20].

Type II interferons

The role of type II IFNs (IFNγ) in the pathogenesis CLE skin injury is not entirely clear. Th1 cells and natural killer (NK) cells stimulated by specific antigens secrete IFNγ. Stimulating keratinocytes with IFNγ increases terminal differentiation and inhibits cell growth by modulating function of B cells, T cells, and macrophages [78]. IFNγ also induces production of the chemokines CXCL9, CXCL10, and CXCL11 [18]. IFNγ and chemokine CXCL10 are increased in discoid lesions [44]. However, blockade of IFNγ decreased CXCL10 but did not change disease activity [79]. More research is needed into the role of IFNγ in CLE.

Type III interferons

Type III IFNs or 'λ' IFNs have been shown to be elevated in the epidermis of CLE lesions [80]. In addition, CLE patients with active skin disease have been shown to have elevated IFNλ that correlated with CXCL9 levels [80]. IFN λ may contribute to skin injury by increasing recruitment of cytotoxic T cells to the epidermis resulting in cellular death and inflammation [81,82]. Further study of type III IFNs in skin injury in SLE is warranted.

Autoantibodies

Autoantibodies and immune complexes are central to tissue injury in SLE [83▪,84], and likely play an important role in the skin. In CLE, immune complex deposition of IgG and IgM at the dermoepidermal junction in lesional and nonlesional skin [85] is considered diagnostic of SLE. The murine model MRL-*Fas*$^{lpr/lpr}$ (also known as MRL/*lpr*) characterized by high levels of autoantibodies, spontaneously develop lupus-like skin injury that appears histologically identical to human SLE and shows a immunofluorescent 'lupus band' [86,87]. Specifically, IgG may be key in skin injury as injection of lupus sera – from either lupus-prone mice or patients with SLE – into the dermis of wild type mice result in an inflammatory response, which was not observed after control serum, isotonic saline, or IgG-depleted sera [88]. Antibodies against galectin 3 and ribosomal protein P0 are related to the development of skin lesions in SLE [89]. Anti-Ro52 is associated

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with an increased risk of photosensitive rash [90], and Ro52 is expressed highly in skin and infiltrating cells after UV-induced skin injury in SLE patients [91,92].

CONCLUSION

The pathogenesis of skin injury in CLE is complex (see Fig. 1), but recent evidence supports a role for dysregulated epidermal cell death. Further research will be needed to understand these molecular pathways to develop targeted treatment modalities and improve disease outcomes.

Acknowledgements

None.

Financial support and sponsorship

G.A.H. was funded in part by the National Institute of Arthritis and Musculoskeletal Diseases of the National Institutes of Health under award T32- AR007197. J.M.K. is supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under awards R01-AR071384, K24AR076975, and P30-AR075043, the Lupus Research Alliance, the Doris Duke Charitable Foundation under a physician scientist development award, the Rheumatology Research Foundation under an Investigator Award, and the A. Alfred Taubman Medical Research Institute and the Parfet Emerging Scholar Award.

Conflicts of interest

J.M.K. has served on advisory boards for AstraZeneca, Eli Lilly, Bristol Myers Squibb, Avion Pharmaceuticals, Provention Bio, Aurinia Pharmaceuticals, and Boehringer Ingleheim. She has received grant funding from Bristol Myerse Squibb/Celgene and Q32 Bio. G.A.H does not have any conflicts of interest.

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New therapeutic approaches in systemic lupus erythematosus

Antonios G.A. Kolios[a,b], *Nobuya Yoshida*[a], *and George C. Tsokos*[a]

Purpose of review
This review gives an overview of the recently published clinical trials in systemic lupus erythematosus (SLE).

Recent findings
Our continuously improving understanding of the cellular and molecular mechanisms, which are involved in the pathogenesis of SLE, has inspired the performance of multiple clinical trials in an attempt to modify recognized targets. Here, we summarize results obtained from recent trials, which used monoclonal antibodies blocking cytokines, blockers of costimulatory molecules or deleting immune cells, small drug inhibitors of kinases and replenishment of cytokines.

Summary
The therapeutic options for patients with SLE grow continuously and in parallel it raises the need for pathogenetic mechanism-based precision medicine so that we may select the right treatment for the right patient.

Keywords
biological therapies, systemic lupus erythematosus, therapy, treatment update

INTRODUCTION

Systemic lupus erythematosus (SLE) is multiorgan autoimmune disease characterized by loss of immune tolerance leading to organ inflammation [1]. The prevalence varies from 20 to 150 cases per 100,000 population. Pathogenesis has been linked to abnormalities in innate and adaptive immune system induced by environmental and hormonal triggers in genetically susceptible individuals [2].

Genetic, environmental and hormonal factors act on various components of the innate and adaptive immune system. Gene copy variants or Single nucleotide polymorphisms influence the expression of many genes involved in the immune response. Environmental factors, including UV light, drugs and products of the microbiome alter T and B-cell responses as well as the function of innate cells by engaging Toll-like receptors. Hormones and genes defined by the X chromosome contribute to disease expression by altering the function of lymphocytes and cells of the innate immune response. All pathways eventually lead to loss of tolerance of B and T cells to autoantigens, which are present in abundance because of increased apoptotic rates of cells and defects in processes responsible for their clearance. The T-cell response to autoantigens is aberrant in terms of early and late signaling events and results in misbalanced levels of cytokines, including decreased IL-2 and increased IL-17 production. T cells also through distinct pathways acquire increased ability to invade tissues and contribute to the inflammatory response. B cells in response to cognate and noncognate (cytokine) interaction with T cells produce autoantibodies. Cells of the innate immune response under the influence of the involved pathogenic factors produce cytokines [including interferon (IFN)-α] or through the direct interaction with lymphocytes contribute in a major way to the inflammatory organ-damaging response. Although several pathways operate in each individual, the relative contribution of each pathway varies from person to person [2].

Clinically, SLE can affect any organ, including the kidney (60%), skin (70%), muscles and joints (85%), the blood (50%), as well as central and

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Curr Opin Rheumatol 2021, 33:181–189
DOI:10.1097/BOR.0000000000000772

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KEY POINTS

- Several promising new treatment approaches have been recently reported in SLE.
- Belimumab showed stable control in long-term extension treatment and anifrolumab (anti-IFN) showed significant improvement in BICLA.
- BIIB059 (plasmacytoid dendritic-cell targeted) showed promising results after a single-infusion.
- Low-dose IL-2 enhances Treg cell function and induces immune tolerance.
- Iguratimod and voclosporin achieved good clinical responses in patients with previously treatment-refractory lupus nephritis.

peripheral nervous system (10%). Constitutional symptoms and fever appear in 70% of patients [3]. Recently, the revised EULAR/ACR classification criteria have introduced antinuclear antibodies as the obligatory entry criterion for the subsequent clinical and immunology criteria, where at least one clinical criterion and ≥10 points are necessary for the diagnosis of SLE [4]. The survival rate improved in the last 70 years from 50% in 4 years to 85% in 15 years; however, there has been almost no additional improvement in the last 30 years and treatment with biologics has been relatively ineffective [5].

The recently updated ACR management recommendations aim the treatment goal for remission or minimal disease activity [6]. Hydroxychloroquine (HCQ), glucocorticoids and small molecules, including methotrexate, azathioprine or mycophenolate mofetil (MMF), represent the commonly used drugs. In people with active disease who do not respond to treatment, the addition of belimumab, rituximab or cyclophosphamide (CYP) is considered.

In this review, we summarize recent reports on clinical trials in people with SLE, which is also based on a better pathogenic understanding of the disease (Figs. 1–3) [7], as well as novel clinical trials (Table 1). As this review is focused on current findings, a full overview is reviewed elsewhere [8].

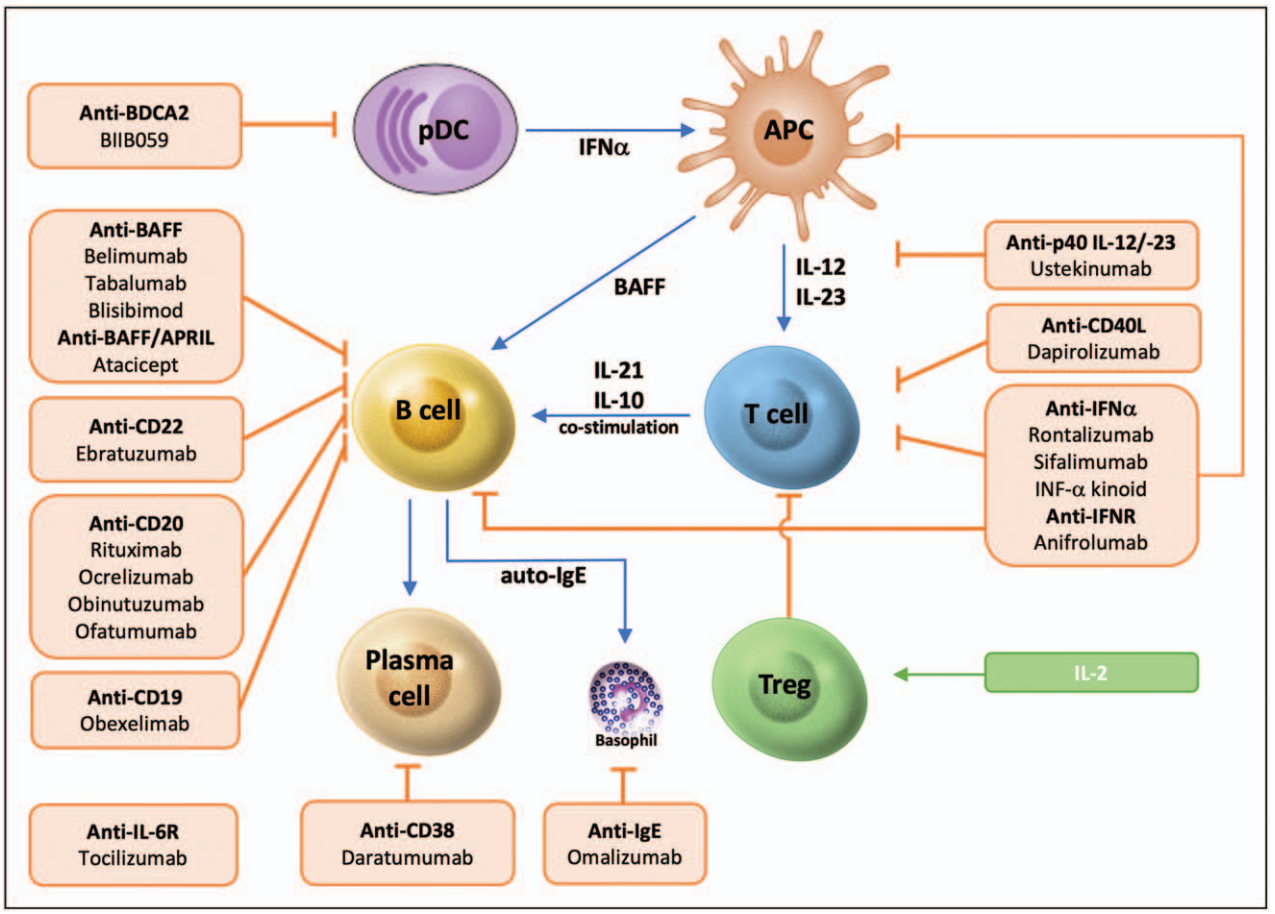

FIGURE 1. Current biologicals in the treatment of SLE or with encouraging results. APC, antigen-presenting cell; IFNR, interferon receptor; JAK, Janus kinase; pDC, plasmacytoid dendritic cell; Treg, regulatory T cell.

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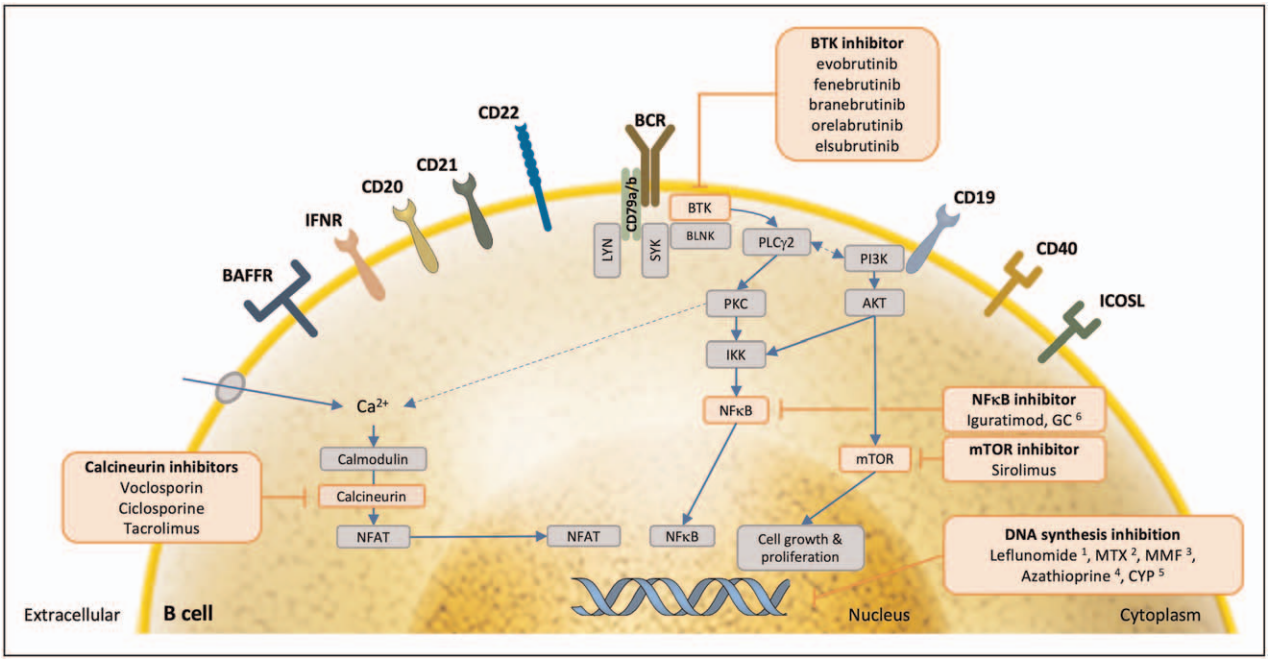

FIGURE 2. Current intracellular B cell targets in human SLE. (1) Inhibition of pyrimidine synthesis and tyrosine kinases; (2) methotrexate, inhibition of AICAR transformylase (low-dose) and dihydro-folate-reductase (high-dose); (3) mycophenolate mofetil, inhibition of IMP dehydrogenase; (4) inhibition of PRPP amidotransferase; (5) cyclophosphamide, alkylating agent; (6) glucocorticoids, inhibition of gene expression of glucocorticoid-responsive elements (e.g. induction of annexin I or MAPK phosphatase 1, direct effects), gene expression of other transcription factors (e.g. NFκB or AP-1, indirect effects) and second-messenger cascades (i.e. the PI3K–AKT pathway). BCR, B-cell receptor; IFNR, interferon receptor; mTOR, mammalian target of rapamycin; NFκB, nuclear factor kappa-light-chain-enhancer of activated B-cells.

METHODS

A PubMed search was conducted for articles published between March 1st, 2019 and September 30th, 2020, using the search MeSH terms 'lupus erythematosus, systemic/therapy' and 'clinical trial' or 'journal article' not 'review' revealed 233 articles, of which 28 articles presenting novel human clinical treatments underwent

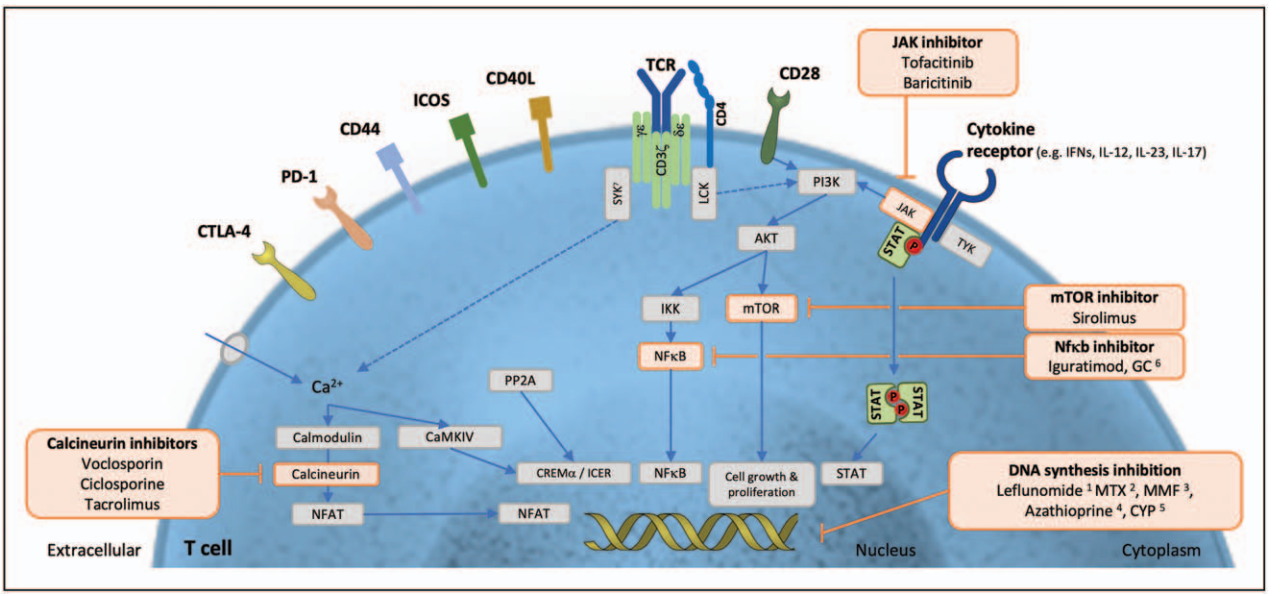

FIGURE 3. Current intracellular T-cell targets in human SLE. (1)–(6) see Figure 2; (7) in SLE SYK is rather than ZAP-70 involved in TCR signaling [7]. IFNR, interferon receptor; JAK, Janus kinase; mTOR, mammalian target of rapamycin; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; TCR, T-cell receptor.

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Table 1. Novel trials in systemic lupus erythematosus

Molecular target	Treatment	Status	Trial registration
B cells			
TACI-Fc fusion protein	RCT-18	Completed phase II study	NCT02885610
		Recruiting phase III study	NCT04082416
ICOSL/BAFF inhibitor	Rozibafusp α (AMG 570)	Recruiting phase II study	NCT04058028
Anti-CD20 mAb	Obinutuzumab	Recruiting phase III study	NCT04221477
Cytokines/Chemokines			
Anti-p19 IL-23 mAb	Guselkumab	Recruiting phase II study	NCT04376827
Anti-IL-21 mAb	BOS161721	Active, not recruiting, phase I/II study	NCT03371251
Anti-IL-17 mAb	Secukinumab	Recruiting phase II study	NCT03866317
	Secukinumab	Recruiting phase III study	NCT04181762
Anti-IL-10 mAb	BT063	Completed phase II study	NCT02554019
IL-2	Aldesleukin	Completed phase II study	NCT03312335, NCT01988506
	ILT-101	Completed phase II study	NCT02955615
	AMG 592	Recruiting phase I study	NCT03451422
	LY3471851 (NKTR-358)	Recruiting phase II study	NCT04433585
Anti-CXCR5 antagonist	PF-06835375	Recruiting phase I study	NCT03334851
Kinases			
BTK	Evobrutinib	Completed phase II study	NCT02975336
	GDC-0853 (fenebrutinib)	Completed phase II study	NCT02908100, NCT03407482
	branebrutinib	Recruiting phase II study	NCT04186871
	ICP-022 (orelabrutinib)	Completed phase I/II study	NCT04305197
BTK and JAK1	elsubrutinib (BTK) and upadacitinib (JAK)	Recruiting phase II study	NCT03978520, NCT04451772
JAK1 and TYK2	PF-06700841	Recruiting phase II study	NCT03845517
JAK1/2	Baricitinib	Recruiting phase III study	NCT03843125
SYK	GSK2646264	Completed phase I study	NCT02927457
TYK2	BMS-986165 (deucravacitinib)	Recruiting phase II study	NCT03920267, NCT03943147, NCT03252587
S1P	ACT-334441 (cenerimod)	Completed phase I/II study	NCT02472795
Various			
Cereblon modulator	Iberdomide (CC-220)	Completed phase II study	NCT02185040
	KPG-818	Completed phase I study	NCT03949426
Anti-CD6 mAb	EQ001 (itolizumab)	Recruiting phase I study	NCT04128579
Anti-MASP-2 mAb	OMS721 (narsoplimab)	Recruiting phase II study	NCT02682407
Anti-C5 mAb	ravulizumab	Not yet recruiting, phase II study	NCT04564339
CB2 agonist	JBT101	Recruiting phase II study	NCT03093402
Artemisinin analog	SM934	Recruiting phase II study	NCT03951259

further investigation. Mouse data were not included.

RESULTS

Targeted therapies/biologicals

Belimumab

In a recent phase III double-blind, randomized, placebo-controlled trial (RCT), 448 lupus nephritis patients received either intravenous (10 mg per kilogram of body weight) belimumab, an anti-BAFF mAb, or placebo, in addition to standard therapy (CYP + azathioprine or MMF) [9▪▪]. At week 104, significantly more patients in the belimumab group reached a primary efficacy renal response, also achieved by more patients at an earlier time point as well as a complete renal response.

In belimumab versus placebo, the risk of a renal-related event or death was lower, ratios of antidouble-stranded DNA or anti-C1q to IgG decreased

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more and urinary protein to creatinine from ≥0.5 to < 0.5 decreased more frequently. The safety profile of belimumab was consistent with previous trials and combination was similar to standard therapy alone. No anti-belimumab antibodies could be detected [9▪▪]. Subcutaneous application of belimumab is currently under investigation in an ongoing phase III study (BLISS-BELIEVE) [10].

Long-term phase III (8 years) and phase IV (13 years) confirmed effectiveness and safety [11,12], as well as a subanalysis of a recent phase II trial in Japanese patients [13]. Additionally, a multicenter, double-blind RCT in African-American patients with active SLE receiving belimumab versus placebo, and standard of care, did not reach the primary endpoint at week 52 for proteinuria in SLE responder index (SRI) response rate in a modified SLEDAI-2K scoring [14].

Anti-interferon mAbs

Anifrolumab, a human type I IFN receptor subunit 1 mAb, showed significant improvement in 352 SLE patients in the BICLA score [British Isles Lupus Assessment Group (BILAG)–based Composite Lupus Assessment] at week 52 as primary endpoint and key secondary endpoints (BICLA response in high type I IFN gene signature patients, reduction in glucocorticoid dose, > 50% skin involvement) in a phase III double-blind RCT (TULIP-2) of intravenous anifrolumab 300 mg versus placebo [15▪▪].

In a double-blind, phase III RCT (TULIP-1) with 457 patients receiving placebo, anifrolumab 150 or 300 mg intravenously every 4 weeks for 48 weeks, the primary endpoint of SLE Responder Index 4 (SRI-4) response was not met, because of with comparable results in placebo versus treatment [16]. Adverse events occurred in both studies in 85–89% in the anifrolumab groups versus 78–84% in the placebo group, whereas serious adverse events (SAEs) were similar among the groups in TULIP-1 compared to 8 versus 17% in anifrolumab versus placebo in TULIP-2.

Sifalimumab, an anti-IFN-α monoclonal antibody, showed long-term efficacy in a recent two-staged phase II open label study in Japanese patients [17▪], similar to previously reported placebo-controlled outcomes [18]. In the current study, adverse events and SAEs were reported in 100 and 57%, respectively, and in both studies, worsening of SLE was the most common SAE with 10–30%, respectively.

Of concern is a recent report [19], which demonstrated that people COVID-19, who developed antibodies to IFN, fared poorly, suggesting that nondiscreet administration of anifrolumab or other anti-IFN mAbs to patients with SLE may compromise their ability to fend off viral infections [20]. In addition to anti-IFN mAbs, caution should be also paid to Interferon-vaccines.

BIIB059

BIIB059 is a humanized mAb targeting the plasmacytoid dendritic cell (pDC)-specific receptor BDCA2 (blood dendritic cell antigen 2) that inhibits type I IFN production and other inflammatory mediators. In a double-blind RCT, BIIB059 was used sequentially in a dose-finding study in healthy volunteers ($n = 54$, part 1) followed by part 2 using a single intravenous 20 mg/kg dose in 12 SLE patients [21▪▪]. Adverse events were mild-to-moderate in 75–88% of healthy volunteer and SLE patients, respectively, and SAEs occurred in one patient in both groups. After BIIB059 administration, expression of IFN response genes in blood decreased, myxovirus resistance protein 1 (MxA) expression normalized and Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) decreased, which was further accompanied by reduced immune infiltrates in skin lesions.

Ustekinumab

Ustekinumab, an antip40 IL-12 /IL-23 mAb, showed persistence over 1 year with a significantly greater efficacy by the SRI-4 at week 24 compared to placebo [22▪▪] in a double-blind, crossover RCT of 102 patients with active SLE as a 1-year follow-up of its previously reported week 24 data [23]. The treatment group received weight-adjusted ustekinumab intravenously (260–520 mg) at week 0, followed by 90 mg subcutaneously every 8 weeks from week 8 on, versus placebo added to standard therapy. After the initial report [23], the placebo group crossed over to ustekinumab 90 mg every 8 weeks at week 24 and increased response index. Safety was comparable to previous ustekinumab indications and patients had significantly lower risk of flares in the ustekinumab-group [22▪▪]. Until week 24 before crossover, the placebo group already showed an improvement because of background therapy. After crossover to ustekinumab, the same improvement like in the ustekinumab group was not seen. In this context, the phase III has been stopped because a planned futility analysis failed to show therapeutic efficacy. The results from the phase-III study are already under evaluation to determine the reasons for the discrepancy with the impressive phase II results.

Interleukin-2

Low-dose interleukin-2 (IL-2), corresponding to doses around 0.3–3.0 million international units daily, stimulates mainly Treg cells and is relatively well tolerated [24]. As either numerical or functional

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Treg cell insufficiency is reported in SLE, several studies in humans used low-dose IL-2 already, including recently either as add-on monotherapy [25▪▪,26▪▪] or in combination with rapamycin [27▪▪]. All studies reported an increase in Tregs. Bystander activation of natural killer cells and eosinophils is reported in low-dose IL-2, without currently obvious negative treatment consequences. Low-to-negative expression of CD25, the IL-2 receptor α, has been reported in SLE patients, with a marked increase upon IL-2 treatment [26▪▪]. Adverse events were rather mild (to moderate), dose-dependent and transient, with most adverse events being injection site reactions, transient flu-like symptoms, including fever and myalgia, or nausea. At the higher end of low-dose IL-2, adverse events also include in higher frequency and severity headaches, dizziness, chills, arthralgia and myalgia [25▪▪,26▪▪,28].

Omalizumab

Auto-IgE antibodies are involved in the activation of pDC and basophils, which induces type I INF responses. Omalizumab, an anti-IgE mAb, showed significant improvement in SLEDAI-2K and reduction in IFN gene signature, especially in IFN-high baseline signature, in 16 patients after 16 weeks of treatment [29▪].

Obexelimab

In a double-blind, phase II RCT of intravenous XmAb5871 (obexelimab), a humanized anti-CD19 antibody Fc-engineered for increased affinity to FcγRIIb, in 104 patients on improving disease activity was included upon withdrawal of immunosuppressives. Only HCQ and glucocorticoids at least 10 mg/day were continued during the study. The primary endpoint, defined as no loss of improvement by day 225, was not significantly different compared to placebo, but time to disease flare was significantly longer in the treatment group [30].

Dapirolizumab

Dapirolizumab pegol is a PEGylated monovalent Fab' antibody fragment with specificity for CD40L, thus blocking costimulation for antigen-presenting cell activation. A double-blind, dose-ranging phase IIb RCT of dapirolizumab in 182 patients with moderately to severely active SLE reported numerically greater improvements relative to placebo across several efficacy endpoints and biomarkers, but BICLA response rate at week 24 as primary endpoint was not met, as not significantly different [31].

Daratumumab

Daratumumab, an anti-CD38 mAb, was recently reported in two patients with life-threatening SLE. Apart from significant transient depletion of long-lived plasma cells, B cells, NK cells and pDCs, also type I IFN activity, T-cell transcripts, SLEDAI-2k score, anti-ds-DNA titer and chronic inflammation were reduced [32▪].

Interestingly, in SLE patients, $CD38^+CD8^+$ T cells bear decreased amounts of cytotoxic molecules associated with increased infection rates [20]. In the current study, single-cell transcriptome analysis revealed that initially impaired cytotoxic function in $CD8^+$ T cells could be restored.

Abatacept

Abatacept, a CTLA4-Ig fusion protein, in combination with glucocorticoid without any further immunosuppressive drugs did not show any difference to the placebo group in a double-blind RCT of 66 SLE patients [33].

Kinase inhibitors

Voclosporin

In a double-blind, phase III RCT of 357 patients with active lupus nephritis (AURORA), voclosporin, a novel calcineurin inhibitor, versus placebo, in combination with MMF and rapidly tapered glucocorticoids, revealed significant renal response at week 52. SAEs were similar between the groups (1 versus 5 deaths in the voclosporin versus MMF group) [34▪].

Tofacitinib

Twice daily treatment with 5 mg tofacitinib, an oral JAK 1/3 inhibitor, for up to a year achieved in a case series of 10 SLE patients complete response in seven and partial response in one patient concerning skin and joint manifestations with significant improvement of SLEDAI-2K and PGA at 3 months [35].

Baricitinib

In a 24-week double-blind, phase II RCT of 314 SLE patients with active skin or joint disease, 2/4 mg daily baricitinib, an oral JAK1/2 inhibitor, versus placebo showed significantly higher resolution of SLEDAI-2K arthritis or rash in the 4 mg baricitinib group. Adverse events were similar between the groups, whereas SAE and serious infections were higher in the 4 mg group [36].

Sirolimus

In a single-arm, open-label, phase I/II trial in patients with active SLE, sirolimus, an mTOR inhibitor, resulted in the reduction of SLEDAI and BILAG disease activity scores at 12 months in 29 patients, who completed the study. Concomitant glucocorticoids could be reduced [37].

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Leflunomide

In an RCT over 24 weeks in 100 Chinese patients with proliferative lupus nephritis low-dose leflunomide (40 mg/day for 3 days, followed by 20 mg/day), an inhibitor of pyrimidine synthesis and tyrosine kinases showed similar complete and partial remission rates for induction treatment as well as adverse events compared to intravenous CYP (0.8–1.0 g per monthly), both in addition to prednisone [38▪].

Immunomodulators

Iguratimod

Iguratimod, an NF-κB inhibitor preventing B-cell differentiation, reducing autoantibody levels and plasma cells, showed promising results with 93% response in an investigational study of 14 therapy-refractory lupus nephritis patients at week 24 [39▪▪].

Lymphokine-based vaccines

In a 36-week phase IIb, double-blind RCT with 185 adults with mild-to-moderate active SLE, an INF-α kinoid vaccine induced neutralizing anti-IFN-α2b serum antibodies in 91% of treated patients and reduced significantly the IFN gene signature. Although the primary endpoint did not statistically differ between treatment and placebo in the modified BICLA response, patients were able to significantly reduce concomitant glucocorticoids. Adverse events in the IFN-K group included infections and injection site reactions with upper respiratory tract infections and arthralgia were thrice more common and nasopharyngitis twice more commen in IFN-K group versus placebo; SAEs were more common in the placebo group [40▪].

Mesenchymal stem cells

In an investigator-initiated trial, 21 treatment refractory SLE patients underwent umbilical-cord-derived mesenchymal stem cell (U-MSC) transplantation, which leads to significant upregulation of peripheral blood $CD1c^{+}$ dendritic cells by expression of FLT3L [41▪▪]. The short-term group of 10 patients at 1-month follow-up showed remarkable improvements in SLEDAI. The long-term follow-up group at 6 months included 11 patients; two patients achieved an SLEDAI complete response, seven a partial response and two progressed. Patients were able to reduce glucocorticoid dose and concomitant immunosuppression. It was previously shown that allogenic U-MSC also shows an immunoregulatory effect in suppressing T cells and B cells, including IgG production, as well as inducing Tregs and macrophage phagocytic activity. Larger studies would need to evaluate the composite biological effect of U-MSC on all cell types.

Ongoing registered clinical trials in systemic lupus erythematosus

Several molecular targets, which are reviewed elsewhere [8,42], are currently under investigation because a relevant association to disease pathogenesis has been claimed. Table 1 summarizes novel treatments in clinical studies, which can either target immune cells directly (e.g. anti-CD20 mAbs and anti-CD6 mAb), relevant cytokines (e.g. IL-23), intracellular pathways (e.g. BTK or JAK), complement system (e.g. anti-MASP-2 mAb and anti-C5 mAb), or cytokine treatments (e.g. IL-2 and muteins).

CONCLUSION

Treatment of SLE is still a challenging endeavor, which is related to its clinical and pathogenetic heterogeneity. A step toward patient-centered precision medicine should require the inclusion of additional diagnostic steps to define the right subset of patients who will benefit from any given biologic/small drug, which targets a specific pathway.

In the last year, several new and promising therapies were developed and much more are awaited in the near future. These interventions either inhibit the inflammatory axis or enhance regulatory immune mechanisms.

As patients with SLE are inherently immunocompromised [43] and most of the novel therapeutics interfere with the required immune response to pathogens, vigilance for possible increased infection rates should be heightened. It was discussed above that IFN mAbs and vaccine may exaggerate the risk for severe COVID-19 [19].

Biologics used in clinical trials should be able to accomplish what they have been prepared for. Yet, there are certain contrivances, which may prevent them to accomplish the expected mechanistic intervention. For example, IL-2 does not trigger a robust signaling response in T cells from people with SLE as it does in normal T cells [44]. B-cell depleting antibodies do not deplete B cells in lupus-prone mice as effectively as in normal mice [45], which suggests that B-cell depletion may not be achieved in all compartments in SLE patients who are administered B-cell-depleting antibodies.

It is apparent that none of the identified pathogenetic pathways is present in all patients with SLE who carry a bona fide clinical diagnosis of SLE.

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Accordingly, targeting of any pathway will benefit only a subset of patients, which may not deliver the expected effect in the study population. Further, it is possible that although a targeted pathway may be operational in a patient with SLE, it may not be the driving pathway and therefore, its correction may not deliver the expected effect both at the immunopathogenic and clinical levels. The need for precision medicine in people with SLE cannot be overstated and is long overdue.

Acknowledgements

None.

Financial support and sponsorship

The work in the Tsokos laboratory is funded by the NIH.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

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Update on the cellular pathogenesis of lupus

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Purpose of review
Aberrations in the innate and in the adaptive arms of the immune system play both important roles in the initiation and progression of systemic lupus erythematosus (SLE). The aim of this study was to provide an update on the most recent findings on the cellular pathogenesis of SLE. Our overview focused particularly on results obtained over the last 18 months.

Recent findings
Recent observations have provided an improved understanding of the importance of low-density granulocytes, a highly proinflammatory subset of neutrophils. We also highlighted in this work recent descriptions of the various cellular sources associated with the interferon signature. In addition, novel contributions have also developed our understanding of the potential importance of extrafollicular T–B-cell interactions in SLE pathogenesis. Finally, the role of recently described B and T-cell subsets, that is, atypical memory B cells, T-peripheral helper cells, and Th10 T cells, were also reviewed.

Summary
Recent findings in the cellular pathogenesis of SLE give a deeper comprehension of previously described mechanisms which drive SLE pathogenesis and shed light on novel players in immune dysregulation that could help to identify potential therapeutic targets.

Keywords
autoimmunity, B cells, systemic lupus erythematosus, T cells, type I interferon

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease, characterized by a loss of tolerance toward nuclear components, leading to autoantibody production, immune complex formation, and multiorgan damage. The pathogenesis of SLE is complex, involving genetic, environmental, and hormonal factors [1]. Important progress has been made over the last decades toward the understanding of the underlying cellular pathophysiology of SLE. Dysregulation of multiple immune cell lineages, belonging to the adaptive but also to the innate immune systems, has been identified as contributor to the expression of systemic autoimmunity. In this review, we will summarize recent insights into the cellular pathogenesis of SLE and provide an update on the most recent findings involving the different cellular compartments of the immune system.

HEMATOPOIETIC STEM AND PROGENITOR CELLS

Hematopoietic stem and progenitor cells are the most primitive multipotent population that give rise to all blood cell types. Their transcriptomic analysis in the NZB/W F1 mouse model of lupus revealed a reprogramming of these cells toward myeloid lineage, with expanded frequencies of common myeloid progenitors [2]. When compared with human SLE hematopoietic progenitor cells, similar data were observed with clear evidence of dysregulated differentiation. Thus, in lupus patients with severe disease, $CD34^{+}$ cells were found to have enhanced proliferation, cell differentiation, and transcriptional activation of cytokines and chemokines that drive differentiation toward myeloid/granulocytic lineage [2]. This inappropriate myeloid versus lymphoid balance may contribute to the

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Curr Opin Rheumatol 2021, 33:190–196
DOI:10.1097/BOR.0000000000000775

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KEY POINTS

- There is a significant heterogeneity amongst SLE LDGs and the majority of them exhibit a high proinflammatory phenotype with proinflammatory effects on T cells.
- Plasmacytoid dendritic cells, which are key producers of type I interferon, can be activated through FcγRIIa receptors and taurine is crucial for their metabolism.
- Extrafollicular T–B-cell interactions appear to be involved in the pathogenesis of SLE, as suggested by data on *Dnase1l3*$^{-/-}$ mice model and by the expansion of T-peripheral helper cells observed in SLE patients.
- In the presence of oxidized mitochondrial DNA, naïve CD4^{+} T can differentiate into Th10 T cells, which provide B-cell help through IL-10 and succinate.

dysregulation of subsequent immune responses particularly during the early biologic phase of the disease, as it could feed the immune system with nuclear-derived antigens.

DYSREGULATION OF INNATE IMMUNITY: ROLE OF NEUTROPHILS

Owing to the presence of self-reactive autoantibodies, SLE has long been considered as predominantly associated with a dysfunction of the adaptive immune responses. However, over the past years, several elements of the innate immune system have been identified as major contributors to the disease pathogenesis [3].

In recent years, several lines of evidence have implicated neutrophils in autoimmune diseases and specially in lupus pathogenesis [4]. Neutrophils of SLE patients exhibit an increased capacity to undergo a specific form of spontaneous cell death, called NETosis, constituting thereby neutrophil extracellular traps (NETs). NETs are networks of decondensed chromatin and associated granular components, histones and cytoplasmic proteins, released into the extracellular space. NETs can induce endothelial damage and favor thrombosis and, harboring autoantigens, they are an important source of autoantigens to trigger autoimmunity. Additionally, SLE patients are characterized by the presence of a specific subset of circulating low-density granulocytes (LDGs), which are phenotypically, functionally and transcriptionally distinct from the other neutrophil subsets. LDGs are highly proinflammatory, as they are important producers of NETs and are able to secrete proinflammatory cytokines (type I interferons [IFN], tumor necrosis factor [TNF]-α, and IFN-γ).

Performing in-depth transcriptomic and epigenetic analysis of SLE LDGs, autologous normal-density neutrophils, and healthy control neutrophils, Mistry *et al.* [5▪] recently identified significant transcriptional and epigenetic heterogeneity amongst SLE LDGs. Thus, they were able to clearly delineate two subpopulations of intermediate-mature and immature neutrophils, identified as LDG^{mat} and LDG^{imm}, characterized by distinct transcriptional profiles and epigenetic landscapes. The majority of SLE LDGs was of intermediate-mature nature, with several functional pathogenic features such as NETs formation, capacity to undergo chemotaxis, and enhanced ability to phagocytosis. This subset, with a high proinflammatory phenotype, could thereby be responsible of the association with organ damage associated with neutrophils.

Another recent study further explored in detail the pathogenic potential of SLE LDGs, with an immunophenotypic, morphological and functional characterization of this neutrophil subset in a well-characterized SLE cohort [6▪]. SLE LDGs, which exhibited heightened surface expression of several activation markers, were found to exert proinflammatory effects on T cells, as their supernatants induced proinflammatory cytokine production (IFN-γ, TNF-α, and lymphotoxin-α) from CD4^{+} T cells. These supernatants did not suppress T-cell proliferation, in contrast to supernatants of normal-density granulocytes. Additionally, LDG prevalence, elevated in SLE patients as compared with healthy controls, was associated with the type I IFN gene signature, as well as with disease activity, as assessed by the SLE disease activity index (SLEDAI) score [6▪]. The association between higher numbers of LDGs with disease activity and low complement levels was further confirmed in another cohort of patients with SLE [7]. Finally, LDGs were also identified as potential biomarkers of cardiovascular risk in SLE patients with subclinical atherosclerosis [8].

Frangou *et al.* [9] explored the exact molecular mechanisms that lead to NETs release in SLE and identified autophagy as a key pathway in this phenomenon. Neutrophils from patients with active SLE exhibited, in the context of proinflammatory microenvironment, increased basal autophagy levels leading to excessive NETs production. Mechanistically, the induction of autophagy was under the dependence of the hypoxia and stress-response protein DNA damage inducible transcript 4/regulated in development and DNA damage responses 1. Exploring the protein composition of SLE NETs, the authors also revealed abundant expression of bioactive tissue factor and interleukin-17A (IL-17A), which promoted thrombin generation and fibrosis, thereby contributing to tissue injury in skin and kidneys [9]. Overall, those studies place LDGs as key contributors to organ

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damage during acute flares, but also to long-term cardiovascular comorbidity.

INTERFERONS

Type I IFNs, which affect multiple components of the immune system, are considered to be key molecules in the pathogenesis of SLE [10]. Overexpression of type I IFN inducible genes, known as the IFN signature, and high concentrations of circulating type I IFN are characteristic of the disease, correlating with several parameters of lupus severity. Mechanistically, a lot of research has been carried out during the last decade and is still ongoing on the identification of the precise cellular sources and mechanisms of type I IFN activity in SLE.

Applying the technique of single-cell RNA sequencing to renal biopsies from patients with lupus nephritis, Der *et al.* [11] confirmed the presence of an overexpression of type I IFN-related genes in lupus patients as compared with healthy controls. Interestingly, this signature was specifically identified in the subset of tubular cells. The renal IFN signature correlated with the one observed in keratinocytes obtained from skin biopsies of the same patients, and predicted the response to lupus nephritis treatment six months after the biopsy, thereby identifying a potential powerful biomarker for this disease.

Plasmacytoid dendritic cells (pDCs) are considered as key producers of type I IFN in SLE. Their activation by nucleic acid-containing immune complexes, via interaction with Toll-like receptors (TLRs), has been well demonstrated, but further studies have tried to identify other pathways involved in their priming. A recent study examined the involvement of Fcγ receptor in this phenomenon [12]. Although IgG-immune complexes appeared to inhibit type I IFN production in healthy individuals by an inhibitory signaling through FcγRIIa on dendritic cells, SLE patients with lupus nephritis displayed an increased responsivity to the stimulation by the same type of immune complexes, resulting in elevated production levels of type I IFN and IL-1β [12]. In addition, a recent study examined the metabolic regulation of type I IFN production by pDCs in the context of autoimmunity [13▪]. Taurine metabolism was found critical in type I IFN production by pDCs. Taurine levels were found higher in SLE patients' sera as compared with healthy controls or rheumatoid arthritis patients, with a positive correlation between taurine content, disease activity, and the expression of IFN signature genes. In lupus-prone mice, taurine supplementation led to a more severe disease with promotion of type I IFN-induced genes expression, lymphocytes activation, increased autoantibodies production, and increased proteinuria [13▪].

Other cellular sources have been pinpointed as associated with the IFN signature. Thus, monocytes were identified as potent producers of IFNα in SLE patients, with a positive correlation between the frequency of IFNα-producing monocytes and SLE-disease activity [14]. Mechanistically, production of IFNα was under the dependency of the cyclic guanosine monophosphate - adenosine monophosphate synthase (cGAS) and stimulator of IFN genes (STING) (cGAS-STING) pathway stimulation. Inhibition of the mechanistic target of the rapamycin (mTOR) pathway downregulated the enhanced STING expression and its downstream molecules and suppressed the subsequent IFNα production by monocytes [14].

Recently, the IFN signature has also been associated with innate lymphoid cells (ILCs), which are immune cells belonging to the lymphoid lineage but which do not express antigen-specific receptors [15]. Alterations in the frequency and in the function of ILCs have been implicated in the pathophysiology of different rheumatic diseases. Blokland *et al.* [15] identified an association between the type I IFN signature in SLE patients and a decreased cell frequency of ILC2 and ILC3 subsets with an elevated expression of Fas (CD95) on their surface.

Type II (IFN-γ) and type III (IFN-λ) IFNs may also play a role in SLE pathogenesis. In a large cohort of SLE patients, Oke *et al.* [16] performed simultaneous measurement of peripheral levels of all three IFN subtypes and demonstrated higher levels in patients as compared with controls (IFN-α, IFN-γ, and IFN-λ1). Interestingly, it was observed different clinical and biological SLE features according to the type of elevated IFN, suggesting that IFNs contribute to the heterogeneity of SLE clinical manifestations. Thus, high type I IFN activity was associated with active mucocutaneous inflammation, whereas high levels of IFN-γ were more associated with nephritis and arthritis [16]. Both type I and type III IFNs appeared to have a possible complementary role in lupus disease pathogenesis [17]. Hiorton *et al.* [17] demonstrated that RNA-containing immune complexes have the capacity to induce type III IFN production by pDCs, especially in the small subset which produces type I IFN as well, connecting thereby the type I and type III IFN production in pDCs and suggesting similar mechanisms at work.

Given its importance in SLE pathogenesis, type I IFN has been considered as a potential interesting target for the development of new therapies. This has been emphasized by the recently published results of the TULIP-2 phase 3 trial, which reported the therapeutic benefit in SLE patients of anifrolumab, a fully human monoclonal antibody targeting type I IFN receptor subunit 1 which inhibits signaling by all type I IFNs [18].

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DYSREGULATION OF ADAPTIVE IMMUNITY: ROLE OF B CELLS

B cells play a central, multifaceted role in the development of SLE, through the production of autoantibodies, production of cytokines, and through their role as antigen-presenting cells [19].

Germinal centers are structures classically developed in the secondary lymphoid organs, but which can also be observed in ectopical sites during autoimmune diseases. It has previously been demonstrated that abnormal germinal center reactions play a crucial role in the development of autoimmunity, particularly by promoting the development of somatically mutated pathogenic autoantibodies. Intriguingly, a recent publication also provided proof that germinal center formation is dispensable for autoreactivity. Using the *Dnase1l3*$^{-/-}$ mice model, Soni *et al.* [20▪▪] demonstrated that autoreactivity against double stranded DNA (dsDNA) is predominantly driven by a T-dependent extrafollicular B-cell differentiation into short-lived plasmablasts. Interestingly, this study underlined also the importance of type I IFN in anti-dsDNA responses, as plasmablasts differentiation and proliferation were driven by type I IFN produced by pDCs.

It has been clearly demonstrated that the B-cell compartment is highly distorted in SLE patients, with important modifications in the proportions and repartition of B-cell subpopulations. Recent studies have examined the role in SLE pathogenesis of a newly described autoimmunity-relevant B-cell subset, named atypical memory B cells (AtMs), characterized as being T-bet^{+}CD11c^{+} and considered as age associated (Table 1) [21]. This population has been known to be expanded in patients with SLE for a long time [22], correlating with disease activity, but their exact role and the mechanisms leading to their generation were poorly defined in the context of lupus. In two cohorts of Chinese patients, Wu *et al.* [23] confirmed the expansion of AtMs in patients with treatment-naïve SLE, correlating with SLEDAI scores, titers of autoantibodies, and complement levels. Lupus AtMs displayed a dysfunctional, apoptosis-prone phenotype. They poorly costimulated T cells to proliferate and produce proinflammatory cytokines. From a transcriptional point of view, these cells exhibited a unique gene expression profile characterized by B cell receptor activation, metabolic dysregulation, and a striking activation of the mTORC1 pathway [23]. Another team unveiled the role of excessive T-bet^{+}CD11c^{+} B cells in aberrant Tfh-cell differentiation [24▪]. Indeed, in the B cell-intrinsic Ship-deficient (ShipΔB) lupus mice model, AtMs compromised the antigen-specific germinal center responses and the process of antibody-affinity maturation, leading to deregulated Tfh-cell responses.

Different studies have recently examined in detail the role of B-cell TLR expression and signaling in the regulation of SLE pathogenesis. Soni *et al.*

Table 1. Newly described B and T-cell subsets and their findings in systemic lupus erythematosus

Population	Differentiation markers	Function	Findings in SLE	References
B cells				
Atypical memory B cells	CD19^{+} T-bet^{+} CD11c^{+}	Exact role in SLE pathogenesis unknown. Involvement in aberrant Tfh-cell differentiation	Expansion in patients with SLE, with correlation of their levels with disease activity Dysfunctional, apoptosis-prone phenotype	[23] [24▪]
CD4+ T cells				
Tph cells	CD4^{+} PD1^{hi} CXCR5^{-}	Promotion of B-cell responses and antibody production outside germinal centers, specifically in sites of peripheral inflammation. Production of important amounts of IL-21	Expansion in the circulation of patients with SLE Presence in kidneys of patients with lupus nephritis Correlation of their levels with disease activity	[34] [35] [36]
Th10 cells	CD4^{+} CXCR5^{-} CXCR3^{+} PD1^{hi}	Promotion of B-cell help through IL-10 and succinate	Amplification in blood and in tubulointerstitial areas of patients with proliferative lupus nephritis Importance of mitochondrial DNA for their generation	[37▪▪]

SLE, Systemic lupus erythematosus; Tfh, T-follicular helper; Tph, T-peripheral helper.

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[20▪▪] demonstrated that anti-dsDNA responses were promoted by TLR9 in *Dnase1l3*$^{-/-}$ mice, in a synergistic and partially redundant way with TLR7. Accordingly, it had been previously demonstrated that TLR9 deficiency led to a loss of anti-dsDNA autoantibodies, but its effects on disease severity have appeared to be opposite. Indeed, in different model of lupus, a protective role of TLR9 has been suggested, as TLR9 deficiency resulted in decreased survival with exacerbated nephritis [25]. To go further in the exploration of these paradoxical results, Tilstra *et al.* [26] evaluated the effects of TLR9 expression in multiple target populations to explore possible cell type specific roles. The effect of TLR9 loss was evaluated using a conditional *Tlr9* knockout (*Tlr9*^{fl}) in B cells (CD19-Cre), dendritic cells (CD11c-Cre), neutrophils (MRP8-Cre), and myeloid cells (both macrophages and neutrophils) (LysM-Cre). Strikingly, only B-cell-specific *Tlr9* deficiency resulted in an acceleration of lupus nephritis and in an alteration of the autoantibody response, whereas its overexpression led to an amelioration of the disease in murphy roths large/*lpr* mice. According to this study, B-cell TLR9 expression appears thereby to be both necessary and sufficient to modulate SLE pathogenesis [26].

Regarding TLR7 expression, it was demonstrated that its high expression in SLE patients, driven by a genetic polymorphism (rs3853839 C/G), was associated with a more active disease and with an upregulation of IFN-responsive genes [27]. Moreover, this overexpression correlated with the expansion of newly formed transitional B cells and promoted autoantibodies production [27].

DYSREGULATION OF ADAPTIVE IMMUNITY: ROLE OF T CELLS

T lymphocytes (CD4$^+$, CD8$^+$, double negative) are key players in SLE disease initiation and maintenance, through the help provided to B cells, the secretion of proinflammatory cytokines and the accumulation of autoreactive memory T cells [28]. The function of the majority of T-cell subsets in SLE have been extensively studied in recent years, with special interest in CD4$^+$ effector proinflammatory subpopulations (such as Th17), in Tfh cells or in T-regulatory (Treg) lymphocytes.

T-regulatory cells

Defects in the CD4$^+$Foxp3$^+$ Treg compartment have been demonstrated in SLE patients. Although most reports agree on reduced numbers or impaired function of circulating Tregs [29], some groups have found increased Treg levels in SLE patients as compared with healthy controls. Thus, in a recent study, examining the phenotype and function of Treg cells in SLE patients, an increase in circulating CD4$^+$Foxp3$^+$ T cells was reported, correlating with disease activity [30]. Interestingly, the increased Treg cells in peripheral blood were mainly derived from thymus Treg, as determined by the completely demethylated status at the Treg-specific demethylated region of the *Foxp3* gene. Functionally, these cells demonstrated a unique dichotomic phenotype, with on one hand, an upregulated expression of IL-17A, and on the other hand, immunosuppressive abilities comparable to that of healthy controls [30]. This feature of Th17 cells in the Treg cell subset was also previously observed by Kato *et al.* [31]. Analyzing CD4$^+$CD25$^+$LAG3$^+$ T-cell subset, which was found significantly increased in SLE patients, authors demonstrated that these cells expressed mRNA of both *FOXP3* and *RORC*, and produced both IL-17 and FOXP3. Yet, they lacked suppressive capacity, in contrast to the subsequent study [30,31].

Concerning the CD8$^+$ Treg cells subset, Deng *et al.* [32] recently demonstrated that the adoptive transfer of CD8$^+$CD103$^+$ regulatory T cells (CD8$^+$CD103$^+$ iTregs) was able in MRL/*lpr* mice, to attenuate glomerular endothelial cell injury, by lowering renal deposition of IgG/C3 and thus reducing renal pathological lesions.

B-cell helper T-cell subsets

During the last decade, numerous studies have provided novel insights regarding the identification of distinct T-cell subsets and their role in providing B-cell help in the context of SLE (Table 1). In the last few months, special interest has been focused on the role of T peripheral helper (Tph) cells in SLE pathogenesis. Tph cells were initially described by Rao *et al.* [33] in 2017 as PD1^{hi}CXCR5$^-$CD4$^+$ T cells, expanded in joints and blood of patients with seropositive rheumatoid arthritis. These cells promote B-cell responses and antibody production outside germinal center, specifically in sites of peripheral inflammation. This population was found, in different cohorts, markedly expanded in the circulation of patients with SLE as compared with healthy controls [34–36]. Tph cells were also identified in kidneys of patients with lupus nephritis, where they correlated with B-cell numbers [35]. Their levels correlated with markers of clinical disease activity, such as SLEDAI score, autoantibodies titers, or complement levels, and they were able to produce important amounts of IL-21 (similar to the ones produced by Tfh cells), a key cytokine for B-cell help [34–36]. The signals important for Tph generation in the context of SLE remain to be discovered.

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Additionally, Caielli *et al.* [37▪▪] also expanded the spectrum of B-cell helper T cells, by the description of a novel $CD4^+$ T-cell population, amplified in blood and in the tubulointerstitial areas of patients with proliferative lupus nephritis. These cells, characterized by the phenotype $CD4^+CXCR5^-CXCR3^+PD1^{hi}$ and designated as Th10 cells by the authors, were distinct from classic Tfh cells and from Tph cells. They did not provide B-cell help through IL-21 but through a unique mechanism involving IL-10 and succinate. Strikingly, the authors demonstrated the importance of mitochondrial DNA (mtDNA) in the generation of this subset. Thus, T cells resulting from coculturing naïve $CD4^+$ T cells with pDCs activated by oxidized mtDNA (a TLR9 ligand) had a characteristic phenotype, distinct from those of T cells originated from cultures with dinucleotide of cytosine and guanine with adenosine-stimulated pDCs (a distinct TLR ligand). The 'oxidized mtDNA T cells' secreted high levels of IL-10, IL-3, and succinate, and were able to induce the differentiation of B cells into plasmablasts [37▪▪].

Altogether, these studies have permitted the identification of new T-cell subsets which drive pathogenic B-cell responses in SLE, especially through extrafollicular T–B-cell interactions. The identification of these subsets may provide interesting new targets from the design of novel therapies.

Double negative T cells

A recent study has elaborated on the molecular mechanisms behind the expansion of αβ $CD4^-CD8^-$ double-negative T lymphocytes in SLE patients [38▪]. Remarkably, the authors demonstrated that loss of splenic marginal zone macrophages, which are usually crucial for establishing immune tolerance, generated an inflammatory milieu, with impairment of apoptotic cells clearance and alteration of the cytokine profile (elevated IL-23, reduced TGFβ). This proinflammatory environment, with self-antigens derived from uncleared cellular debris, provoked in turn the activation, expansion and survival of double-negative IL-17 producing T cells, resulting from the conversion of self-reactive $CD8^+$ T cells [38▪].

CONCLUSION

SLE is a heterogeneous disease with a complex pathogenesis, which is still far away to be fully understood. Dysregulated immune responses have been extensively studied and within the last few months, new insights have been obtained on innate and adaptive immune cells' abnormalities, especially regarding the role of neutrophils, type I interferon, B cells, and T cells. A clearer comprehension of the mechanisms driving SLE pathogenesis will help to identify potential safer and more effective treatments.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

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Cellular aspects of the pathogenesis of lupus nephritis

Anthony Chang[a], *Marcus R. Clark*[b], *and Kichul Ko*[b]

Purpose of review
Lupus nephritis is a common severe manifestation of systemic lupus erythematosus. Despite recent advances in therapeutics and understanding of its pathogenesis, there are still substantial unmet needs. This review discusses recent discoveries in these areas, especially the role of tubulointerstitial inflammation (TII) in lupus nephritis.

Recent findings
Non-white ethnicity is still a major risk and poor prognostic factor in lupus nephritis. TII and fibrosis have been found to be associated with worse renal outcome but the current lupus nephritis treatment guidelines and trials are based on the degree of glomerular inflammation. In combination with mycophenolate mofetil, a B-cell-targeted therapy (belimumab) and a calcineurin inhibitor (voclosporin) have shown efficacy in recent lupus nephritis trials. However, response rates have been modest. While lupus glomerulonephritis results from immune complex deposition derived from systemic autoantibodies, TII arises from complex processes associated with *in situ* adaptive cell networks. These include local antibody production, and cognate or antigen-induced interactions between T follicular helper cells, and likely other T-cell populations, with antigen presenting cells including B cells, myeloid dendritic cells and plasmacytoid dendritic cells.

Summary
Better understanding of the pathogenesis of TII will identify novel therapeutic targets predicted to improve outcomes in our patients with lupus nephritis.

Keywords
adaptive immunity, lupus nephritis, pathogenesis, tubulointerstitial inflammation

INTRODUCTION

Lupus nephritis is one of the most common severe manifestations of systemic lupus erythematous (SLE), occurring in up to 50% of SLE patients during the course of the disease [1,2,3▪,4▪,5,6]. Lupus nephritis occurs in racial/ethnic minorities at increased frequency in the United States [1,3▪,7], and this trend has also been found in pediatric population [8] as well as in other parts of the world [4▪,8]. Despite the dramatic renal survival improvement in lupus nephritis in the 1970–1980s, this has not changed much since then [9–11]. Still up to 50% of lupus nephritis cases progress to end-stage renal disease (ESRD) [10,12], and lupus nephritis, especially when it results in ESRD, is one of the most important predictor of mortality in SLE [6,13,14]. There is an urgent need to better understand the disease pathogenesis and develop new therapeutic approaches.

In this review, we discuss the new discoveries in pathogenic mechanisms in lupus nephritis especially those involving the tubulointerstitial space in kidneys along with recent clinical trials in lupus nephritis.

DEMOGRAPHIC AND SEROLOGICAL PREDICTORS OF POOR PROGNOSIS

In addition to non-white race/ethnicity, younger age at the time of SLE diagnosis is another risk factor for development of lupus nephritis [2,7], along with male sex [6,15] although this difference between sexes may not be seen in all ethnic groups [16]. The majority of lupus nephritis cases occur either

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Curr Opin Rheumatol 2021, 33:197–204
DOI:10.1097/BOR.0000000000000777

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KEY POINTS

- A B-cell-targeted therapy, belimumab and calcineurin inhibitor, voclosporin have shown success in recent clinical trials for lupus nephritis.
- TII and fibrosis are poor prognostic factors in lupus nephritis.
- TII is associated with *in situ* adaptive immunity which is distinct from systemic autoimmunity which is related to glomerular process in lupus nephritis.

at or in the first 5 years of SLE diagnosis, and the time to lupus nephritis development is also shorter in non-white patients [2,3▪,17].

Lupus nephritis patients with non-white ethnicities especially those with African ancestry have a worse renal prognosis [12,18,19], and these findings may be due to both biological and socioeconomic factors [18–20]. Serologically, positive anti–Sjögren's-syndrome-related antigen A antibodies [12], low complements [18] and high-serum creatinine [10,12] correlate with poor renal outcome.

HISTOLOGY AND CLASSIFICATION OF LUPUS NEPHRITIS

The framework of the lupus nephritis classification has always centered around glomerular alterations and first was formalized under the auspices of the World Health Organization in 1974. Refinements to the classification have occurred in every subsequent decade and the latest 2018 International Society of Nephrology/Renal Pathology Society (ISN/RPS) version maintains most of the original framework [21]. Mesangial immune complex deposition depending on its severity is diagnosed as minimal mesangial (Class I) or mesangial (Class II) lupus nephritis. The presence of active or chronic glomerular lesions are diagnosed as focal (Class III) or diffuse (Class IV) lupus nephritis. Membranous (Class V) lupus nephritis (mLN) can be present in conjunction with class III or IV lupus nephritis. When membranous and mesangial lupus nephritis are both present, the class V diagnosis supersedes class II. End-stage (Class VI) lupus nephritis represents the presence of more than 90% global glomerulosclerosis.

The following are active glomerular lesions: Glomerular basement membrane (GBM) break, fibrinoid necrosis, cellular or fibrocellular crescent, endocapillary hypercellularity, prominent immune complex deposition in the form of 'wire-loop' or hyaline pseudothrombi, or karryorhexis. The rupture of the GBM leads to fibrinoid necrosis and crescent formation. The remaining active lesions are related to immune complex deposition or the inflammatory response to these glomerular immune complexes.

Given that the lupus nephritis classification categories are fairly crude, the addition of the National Institute of Health (NIH) activity and chronicity indices provides additional granular information regarding the degree of active and chronic lupus renal disease, which is pertinent for focal and diffuse lupus nephritis [21]. For example, a lupus nephritis biopsy with 5 or 45% active glomerular lesions would both be considered focal lupus nephritis. Likewise, a biopsy with 50% compared with 90% active glomerular lesions would both be considered diffuse lupus nephritis.

The NIH activity index considers the following six parameters: Cellular and/or fibrocellular crescents, fibrinoid necrosis, wire-loop and/or hyaline 'thrombi,' neutrophils/karyorrhexis and interstitial inflammation. These individual parameters are assessed and the extent of glomerular involvement is determined using these categories of 0, 1–24, 25–50 or more than 50%, which are assigned a semiquantitative score of 0–3 points. The cellular/fibrocellular crescent and fibrinoid necrosis scores are both multiplied by two for a score of 24 total points. The NIH chronicity index assesses four parameters: Global glomerulosclerosis, fibrous crescents, tubular atrophy, and interstitial fibrosis and a sum of these four categories (0–3 points for each) is a score of 12 total points.

The extent of activity reflects ongoing injury that may be responsive to therapeutic intervention. Chronicity indicates scarring and irreversible damage that is unlikely to respond to therapy.

THE PROGNOSTIC IMPORTANCE OF TUBULOINTERSTITIAL INFLAMMATION AND TUBULOINTERSTITIAL SCARRING

Tubulointerstitial inflammation (TII) repeatedly has been demonstrated to be an important pathologic parameter, but remains overshadowed by the glomerulocentric approach to the clinical management of lupus nephritis. Hill *et al.* [22] first identified interstitial inflammation as 'one of the pivotal variables' in lupus nephritis which has been confirmed in several more recent studies. In fact, we found that TII was more predictive than the glomerular injury [23].

The degree of interstitial fibrosis and tubular atrophy (IF/TA) is the pathologic parameter that best predicts clinical outcomes in lupus nephritis [23,24,25▪▪], and this is true for many renal diseases. In fact, tubulointerstitial scarring was more

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important than the other three glomerular parameters that were ultimately included in the Oxford IgA nephropathy classification. Of note, interstitial inflammation was also a significant pathologic parameter, but did not provide any information beyond tubulointerstitial scarring so it was eliminated from the final classification [21]. While this was implemented for practical purposes, the omission of TII in IgA nephropathy has a similar inadvertent effect of shifting attention back to glomeruli.

African ancestry which is one of the most important predictors of poor renal outcome has been shown to be associated with presence of moderate to severe TII which in turn correlates with presence of IF/TA [23,26]. Therefore, current classifications largely fail to capture the prognostically important histological features which are especially frequently found in lupus nephritis patients with African ancestry.

RECENT CLINICAL TRIALS WITH LUPUS NEPHRITIS

Treatment of lupus nephritis has made incremental progress in the past few decades. Despite the tubuloinsterstitial process being an important prognostic factor, patients with lupus nephritis continue to be stratified based on the glomerulocentric ISN/RPS classification criteria [21] for therapeutic strategies to be determined [27▪▪,28]. For proliferative lupus nephritis (pLN, Classes III and IV) either with or without mLN (Class V), the induction regimen traditionally had involved intravenous cyclophosphamide (CYC) with glucocorticoids based on the NIH trials [29]; however, in the past 2 decades, low-dose CYC and mycophenolate mofetil (MMF) have emerged as noninferior but safer alternatives [30–33]. In fact, MMF has shown higher efficacy over monthly intravenous CYC in pLN patients, especially in groups mostly comprised of African ancestry or Hispanic ethnicity [31,32]. MMF and azathioprine are both the mainstay medications for pLN maintenance after induction [28,34,35], whereas MMF has shown superiority over azathioprine in one international study [34].

Pure mLN cases have often been included in small numbers in the previous large trials for lupus nephritis [29–33], and thus, data for pure mLN treatment are limited. Both the American College of Rheumatology and European Union League Against Rheumatism recommend MMF along with glucocorticoids as an induction therapy for pure mLN [27▪▪,28]. Of note, CYC, MMF and azathioprine are all used as off-label therapies in lupus nephritis.

Numerous other therapies have been studied in lupus nephritis and most failed to show positive results [36,37]. Despite many setbacks in recent years, different medications especially in combination against various targets are rigorously being researched, and are starting to demonstrate promising results. Tacrolimus, a calcineurin inhibitor, either alone or in combination with MMF has shown efficacy in lupus nephritis in Chinese patients [38,39]. Voclosporin, another calcineurin inhibitor which is not yet approved by the United States Food and Drug Administration, in combination with MMF showed superiority over MMF alone in phase 2 and 3 multiethnic trials [40▪▪,41▪▪]. Despite the failure of rituximab, a monoclonal anti-CD20 antibody in a large randomized controlled trial for lupus nephritis [42], another B-cell-targeted therapy, belimumab which targets B-cell activating factor showed positive results in a phase 3 trial in combination with MMF [43▪▪]. While the successful results from these novel approaches are encouraging, the renal response rates are still well below 50%, and their efficacy is unclear in patients of African ancestry due to the small size of this cohort in the trials [40▪▪,41▪▪,43▪▪]. Therefore, there are great unmet needs in lupus nephritis research.

LUPUS GLOMERULONEPHRITIS AS A MANIFESTATION OF SYSTEMIC AUTOIMMUNITY

Lupus nephritis is often equated with glomerulonephritis. Canonically, lupus glomerulonephritis is driven by immune complex-mediated inflammation in which autoantibodies and preformed immune complexes deposit in glomeruli and cause inflammation. Much work has focused on the role of anti-double stranded (ds) DNA antibodies. Indeed, serum titers of anti-dsDNA antibodies correlate with proliferative glomerulonephritis [44]. Elegant studies have demonstrated that anti-dsDNA antibodies can form immune complexes with DNA-wrapped nucleosomes, and these can then directly deposit in glomeruli [44–46]. Alternatively, anti-dsDNA antibodies can directly bind glomeruli. This could be because of cross-reactivity, or recognizing DNA bound to collagen or chromatin fragments bound to lamin and collagen [45]. Anti-dsDNA antibodies have been purified from nephritic kidneys [47,48]. Importantly, infused anti-dsDNA antibodies can bind glomeruli and induce glomerulonephritis in nonautoimmune mice [49,50]. This provides a direct pathogenic link between anti-dsDNA antibodies and glomerulonephritis. Significantly, not all anti-dsDNA antibodies are likely to be nephritogenic [51]. Therefore, there must be specific biophysical features, beyond just binding dsDNA, that confer pathogenicity.

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More recent work has implicated other immunological mechanisms in lupus glomerulonephritis. Among these are neutrophils and their ability to extrude DNA/protein nets [52–54]. Oxidized mitochondrial DNA released by neutrophils and other cells, also drives local inflammation [55].

Furthermore, lupus is associated with increased circulating levels of an activated subset of neutrophils, low-density granulocytes (LDGs), that generate nets (NETosis) more easily than other neutrophil populations [56▪,57▪]. These LDGs, presumably, provide for abundant nets that drive systemic inflammation and breaking of both B and T-cell tolerance. Neutrophils respond to multiple inflammatory stimuli, including interferons and autoantibodies, which can make them more likely to undergo NETosis. Therefore, it is unclear if neutrophil dysregulation is a secondary manifestation, or primary cause, of lupus. They might be more important is amplification of systemic inflammation rather than in initiating disease. Furthermore, in mice, neutrophils can also repress inflammation and B-cell activation [58] and neutrophils play multiple homeostatic roles [52]. This suggests the contributions of neutrophils to lupus might be complex.

Recent transcriptomic studies of peripheral blood have revealed seven different lupus subsets [59]. Most notably, a predominance of neutrophil transcripts, a neutrophil signature, correlated with active nephritis. However, neutrophils are not a characteristic histologic feature of lupus nephritis. Indeed, they are only occasionally seen in very inflamed glomeruli and are essentially absent from the tubulointersititum (discussed below). Therefore, it is likely that neutrophilia and NETosis are feature of systemic autoimmunity that contribute to a general inflammatory state and loss of systemic tolerance.

There is also a relationship between lupus activity, titers of anti-dsDNA antibodies and circulating levels of T follicular helper (Tfh) cells [60,61]. Tfh cells are specialized for providing help to B cells in germinal center light zones [62]. Indeed, it has been demonstrated in mice that Tfh cells help limit and direct somatic hypermutation and affinity maturation [63]. In human peripheral blood, at least three different Tfh-like populations have been detected that differ in their in-vitro activities [64]. However, none of these populations are directly comparable with those Tfh cells in germinal centers. While correlated with lupus activity, Tfh cells are not observed in glomeruli [65]. However, as discussed below, they might play an important role in TII.

Indeed, for all the peripheral immune mechanisms that have been associated with lupus glomerulonephritis, the histological findings in glomeruli are rather nonspecific and consist of mostly effector T cells and macrophages. We propose this reflects systemic autoimmunity and a linear pathogenic model. In other words, neutrophils, Tfh cells, plasmacytoid dendritic cells (pDCs) and associated processes contribute to glomerulonephritis by breaking tolerance and inducing inflammation. Then, the products of autoimmunity and inflammation are exported to glomeruli in the form of T cells and antibodies. In this model, there are no amplification loops that involve those processes active in glomeruli.

TUBULOINTERSTITIAL INFLAMMATION ASSOCIATED WITH IN-SITU ADAPTIVE CELL NETWORKS

In contrast to glomerular inflammation, inflammation in the tubulointerstitium is complex and in many cases organized into structures reminiscent of those observed in secondary lymphoid organs [66]. In an earlier study, closely packed T : B aggregates were observed in about half of patients while five of 70 had germinal center-like structures including clearly formed light and dark zones, follicular dendritic cell networks and discrete areas of proliferating B cells [67]. Indeed, sampling of these germinal centers using laser capture microscopy and extensive sequencing revealed strong clonal expansion and ongoing somatic hypermutation. Given that these were diagnostic needle biopsies, providing a very small sample size, we likely underestimated the prevalence of tertiary lymphoid neogenesis. These data clearly demonstrate *in situ* antigen-driven selection which has not been observed, and indeed is unlikely to occur, in inflamed glomeruli.

Furthermore, it is likely that a restricted number, and classes of antigens, drive *in situ* B-cell selection in lupus TII. Indeed, cloning and expressing antibodies expressed by clonally expanded intrarenal B cells revealed that the majority expressed antibodies that bound cytoplasmic, ubiquitously expressed antigens [68]. Among these, most directly bound vimentin. In contrast, across eight patients we did not find clonal expansion of B cells expressing anti-dsDNA antibodies.

Vimentin is an intermediate cytosolic filament and has been thought to be a structural protein. However, mice with a deletion in the gene encoding vimentin are phenotypically normal [69]. Furthermore, vimentin is strongly upregulated by some inflammatory and injured cells. Indeed, vimentin is highly expressed throughout the inflamed lupus tubulointerstitium [68]. In activated macrophages, vimentin is secreted and presented on the cell surface suggesting roles other than that of a structural

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protein [70]. Furthermore, vimentin might be a proinflammatory molecule sensed by Dectin-1 [71]. These data suggest that tolerance is broken *in situ* to molecular patterns of inflammation. This provides a potential *in situ* feedforward mechanism in which inflammation elicits local adaptive immunity leading to antibody deposition and more inflammation.

In a cross-sectional cohort, serum anti-vimentin antibodies (AVAs) correlated with TII severity [72▪]. Furthermore, high-titer AVAs in lupus nephritis patients predict a poor response to both MMF and MMF and rituximab therapy. Significantly, AVA serum titers did not correlate closely with other autoantibodies and, in contrast to anti-dsDNA antibody titers, did not change substantially with therapy. These data suggest that serum AVAs provide a measure of TII in the periphery that is prognostically meaningful and different than that provided by other antibody specificities. We would propose that while serum anti-dsDNA antibodies reflect mechanisms relevant to glomerulonephritis, AVAs capture a TII pathogenic process. In the periphery, selection is for antibodies to DNA or RNA protein complexes. Work in mice has shown these specificities to be dependent on Toll-like receptor signaling [73]. In contrast, vimentin is a protein antigen to which B cell responses should be fully dependent upon T-cell help.

Indeed, in addition to B cells, there are Tfh cells within the inflamed tubulointerstitium [65]. These Tfh cells are mature, with high levels of IL-21 indicating they have recently provided productive help to B cells. Furthermore, these intrarenal Tfh cells are in intimate contact with B cells forming complex immunological synapses consistent with ongoing cognate help. These data suggest that in addition to recognizing antigen, intrarenal B cells are getting critical costimulation from cognate T cells. These two signals are predicted to provide the necessary stimulation for full *in situ* activation and differentiation. Indeed, our analysis indicates that the T : B aggregates that are often observed histologically represent collections of Tfh cells providing help to B cells. Significantly, within these aggregates, there is relatively little proliferation (unpublished observation). Rather, proliferation is seen within the intrarenal plasmablast pool. We propose that there is selection within T : B aggregates for cells that subsequently differentiate into plasmablasts.

Different Tfh-cell populations have been identified in lupus. Notable is a population of T cells in peripheral blood, and in the inflamed tubulointerstitium, that can activate B cells via IL-10 and succinate [74▪]. In contrast to canonical Tfh cells, these cells do not rely on IL-21 for B-cell activation. In rheumatoid synovium and the blood of lupus patients, peripheral T helper cells have been described which differ from Tfh cells in the chemokine receptors they express and possibly in their underlying molecular programming [75,76]. Therefore, the heterogeneity of T-cell helper pools across blood and tissue, and their relative importance in activating B cells in different diseases, remain to be understood.

SLE is thought to be the canonical B-cell-driven systemic autoimmune disease and therefore much effort has been directed to defining how autoreactive B cells are selected and activated. However, recent single cell (sc) RNA-Seq experiments have revealed great complexity in the cells infiltrating the lupus kidney including populations of CD8+ T cells, natural killer cells, conventional dendritic cells, macrophages and pDCs [77▪▪]. In another related study that captured both immune cells and renal tubular/stroma cells, it was clear that important ligand/receptor pairs mediate communication between immune cells and their environment [78▪▪]. This complex web of interactions likely drive *in situ* inflammation and fibrosis. These studies were done on relatively few biopsies and the data were reported in aggregate. We do not know if all cells are present in all biopsies. Furthermore, we do not know the spatial or functional relationships between these different cell populations. Unraveling how these cells independently, and as part of immune cell networks, mediate TII and fibrosis will almost certainly reveal new therapeutic targets.

These scRNA-Seq studies also provided insights into the phenotypes of the B cells infiltrating the inflamed kidney. Notably, double negative (DN, CD27-IgD-) B cells appeared common [77▪▪]. These cells have been studied in the periphery, accumulate with age and have been associated with autoimmunity [79,80]. One of the markers they express is the transcription factor, T-bet, which most commonly lies downstream of Toll-like receptor activation [81]. Therefore, DN cells likely arise from activation pathways commonly implicated in lupus including those involved in the generation of anti-dsDNA and antiribonucleoprotein antibodies. However, the antibody repertoire of these DN cells is not known. It will be important to resolve intrarenal B cell heterogeneity and to determine if DN cells, or other *in situ* B-cell populations, express AVAs.

The most common lymphocyte population in the kidney are CD4+ T cells. While some of these are Tfh cells, the majority are not (unpublished observation). The role of these non-Tfh CD4+ T cells is still not clear. However, at least one mechanism by which they are activated *in situ* has been identified. pDCs are canonically primary sources of interferon alpha

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(IFNα). However, it is now clear that pDC subsets can present antigens [82]. Significantly, it appears that antigen presentation and IFNα secretion by pDCs are mutually exclusive states; at any one time, a pDC does one or the other. However, single-cell studies have demonstrated that the same cell can, over time, do both functions [83▪]. In recent studies, we have used confocal microscopy and deep machine learning to demonstrate that pDCs are important *in situ* antigen presenting cells (APCs) in lupus nephritis [84▪▪]. Indeed, in most patients studied, they were more important APCs than classical CD11c+ dendritic cells. Significantly, these cells did not express markers of antigen presenting pDCs in the periphery. Therefore, the relationship between peripheral and intrarenal pDCs is unclear. Furthermore, how *in situ*, pDCs form different functional populations is not known. There is a great deal to learn about the *in situ* APCs in lupus nephritis.

The above results demonstrate that many cell types, and many immunological mechanisms, mediate lupus TII. Furthermore, they appear to be quite different than those associated with lupus glomerulonephritis. This includes both the cells involved and the antigen specificities driving lymphocyte selection in each renal compartment. At the time of clinical presentation, glomerular and TII are markedly different. However, inflammation in each compartment might arise from similar initiating mechanisms. Indeed, we propose that a systemic autoimmune diathesis is likely required for TII to develop. Furthermore, inflammation in the two renal compartments could be functionally related. Indeed, tubulointerstitial hypoxia, which has been related to glomerular inflammation [66], is a feature of both human and mouse lupus TII [85].

The vast majority of lupus therapies are predicated on a model of systemic autoimmunity whose relevance might be limited to glomerulonephritis. To reveal new therapeutic targets, that are likely to alter the natural history of lupus nephritis, it will require identifying important intrarenal mechanisms driving TII, fibrosis and ultimately renal failure.

CONCLUSION

Despite the recent scientific advances in lupus nephritis, there is still substantial room for improvement. Currently, the focus of lupus nephritis, both in clinical trials and mechanistic studies, is still focused on lupus glomerulonephritis rather than TII. As described in this review, there is a tale of two pathogenic mechanisms in lupus nephritis, one involving the glomeruli and systemic autoimmunity, and the other involving the tubulointersitium and *in situ* local adaptive immunity. Understanding the pathogenesis of TII and tubulointerstitial scarring will reveal new and better therapeutic targets which will diminish mortality and improve the quality of life for those afflicted with lupus nephritis.

Acknowledgements

None.

Financial support and sponsorship

M.R.C. gets support from grants from the National Institute of Health (U19 AI082724) and Department of Defense (LRI180083), both of which are relevant to this article.

Conflicts of interest

A.C. is a consultant and on the speaker's bureau for Alexion Pharmaceuticals Inc.

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New lupus criteria: a critical view

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Purpose of review
To review the validation of the European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) 2019 classification criteria for systemic lupus erythematosus (SLE).

Recent findings
Positive antinuclear antibodies, which constitute the obligatory entry criterion of the EULAR/ACR criteria, were found in the vast majority of SLE patients worldwide, with 97% (94–100%) of patients antinuclear antibodies positive in studies investigating EULAR/ACR criteria performance. Combined over the publications, EULAR/ACR criteria sensitivity was 92% (range 85–97%). Specificity varied more relevantly, with the publications published after the EULAR/ACR 2019 criteria showing 93% (83–98%) specificity. Of particular relevance is the good performance of the EULAR/ACR criteria seen in pediatric SLE as well as in early SLE.

Summary
The new classification criteria have been investigated in an impressive number of cohorts worldwide, adding to the data from the EULAR/ACR criteria project cohort. It is critical to strictly keep to the attribution rule, that items are only counted if there is no more likely alternative explanation than SLE, the domain structure, where only the highest weighted item in a domain counts, and the limitation to highly specific tests for antibodies to double-stranded DNA.

Keywords
classification criteria, cutaneous lupus erythematosus, pediatric systemic lupus erythematosus, systemic lupus erythematosus

INTRODUCTION

One year has passed since the publication of the European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) 2019 classification criteria for systemic lupus erythematosus (SLE) [1,2]. The new SLE criteria have introduced some structural changes. First, antinuclear antibodies (ANA) were shifted to the position of an obligatory entry criterion. Second, criteria items were weighted, with weights ranging from 2 to 10. Third, the items were ordered in domains, only the highest weighted out of each is counted. Fourth, individual exceptions for singular items were replaced with one attribution rule that excludes items from counting that are more likely explained by another cause than SLE. Two rules have not changed from the previous criteria sets [3–5], but may still need reiteration: All criteria count if they have ever been fulfilled, that is, historical items and items not present at the same time are still fully honored. Second, at least one clinical criterion is needed, which is also constituted by hematology criteria.

A goal of the EULAR/ACR classification criteria project was to increase sensitivity as compared with the ACR criteria, but maintain specificity. This was based on data that the Systemic Lupus International Collaborative Centers (SLICC) group 2012 criteria had increased sensitivity, but at the same time lost specificity, at least when compared with relevant disease controls [6]. In the validation cohort, the EULAR/ACR 2019 classification criteria achieved a sensitivity of 96% and a specificity of 93%, which favorably compared with the ACR 1997 criteria (83% sensitivity, 93% specificity) and the SLICC 2012 criteria (97% sensitivity, 84% specificity). A subanalysis on SLE patients by ethnicity, sex, and

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Curr Opin Rheumatol 2021, 33:205–210
DOI:10.1097/BOR.0000000000000771

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KEY POINTS

- Additional analyses of the EULAR/ACR classification criteria have found good performance across ethnicities, in men and in early SLE.
- Groups worldwide have externally validated the new criteria. Overall, the performance characteristics are in line with the validation data of the project.
- Of particular importance is the external validation in pediatric and juvenile patients, who were not represented in the EULAR/ACR classification criteria cohorts.

early disease confirmed that the EULAR/ACR 2019 criteria performed well, with improved sensitivity as compared with the ACR criteria and improved specificity as compared with the SLICC criteria over several subsets [7▪].

PERFORMANCE WORLDWIDE

Groups worldwide have compared the performance of the EULAR/ACR 2019 criteria to the performance of the ACR 1997 and SLICC 2012 criteria sets [8–10,11▪▪,12,13,14▪–16▪,17,18]. In most of these studies, the sensitivity was higher than the sensitivity of the ACR criteria (Table 1). Specificity was more variable, and appeared particularly low in the studies published before the EULAR/ACR criteria were published [9,17,18]. There are indications that the EULAR/ACR criteria common attribution rule in particular was not followed in some of the studies, which could explain lower than expected specificity for the EULAR/ACR criteria. In addition, criteria dependent inclusion of patients into cohorts may have biased against the new criteria in some instances. Reassuringly, improved specificity as compared with the SLICC criteria was also found for the SLICC validation cohort [11▪▪].

Two studies using Latin American cohorts, namely on the Grupo Latino Americano De Estudio de Lupus (GLADEL) [19] and the Lupus in Minorities: Nature versus Nurture (LUMINA) [20] cohorts have not directly reported sensitivity and specificity data. They have instead focused on the timepoints when the various classification criteria were met. The GLADEL cohort recruited patients by SLE diagnosis, but more than 95% met ACR criteria. Of 1480 patients, 49 (3.3%) were excluded because of having negative ANA and 68 (6.5%) because they did not meet EULAR/ACR 2019 criteria within 6 months after having met ACR and SLICC criteria [19]. 58% met the EULAR/ACR and ACR criteria at the same time, while 7% met the EULAR/ACR criteria and 34% met the ACR criteria earlier, with mucocutaneous items and white origin associated with meeting the new criteria later. SLICC and EULAR/ACR criteria were met at the same time by 70.8% of patients, with most of the others (28.6%) meeting SLICC criteria earlier, with alopecia, positive antibodies to double-stranded DNA (dsDNA) and positive antiphospholipid antibodies being associated

Table 1. Performance of the three systemic lupus erythematosus classification criteria sets in recent studies

First author	SLE patients			Non-SLE	EULAR/ACR criteria		ACR criteria		SLICC criteria	
		n	ANA%	*n*	Sens%	Spec%	Sens%	Spec%	Sens%	Spec%
Aringer	Validation	696	99	574	96	93	83	93	97	84
Adamichou	Greece	690	94	401	89	97	86	93	91	94
Dahlström[a]	Sweden	56	98	56	93	[a]73	83	82	100	75
Lee	Korea	335	99	337	98	91	96	94	99	93
Petri	US	293	98	423	91	89	83	96	97	84
Rubio	US	217	96	0	94	NA	94	NA	100	NA
Suda	Japan	100	97	0	[b]92	NA	97	NA	99	NA
Teng	China	199	99	175	97	90	75	96	92	84
Aljaberi	Pediatric	112	96	105	85	83	72	87	NA	NA
Ma	Pediatric	156	100	379	97	98	87	100	97	100
Rodrigues[a]	Pediatric	122	[c]100	89	88	[a]67	71	83	89	91
Gegenava[a]	NPSLE	294	96	66	87	[a]74	89	89	85	76

ANA, antinuclear antibodies; EULAR/ACR, European League Against Rheumatism/American College of Rheumatology; SLE, systemic lupus erythematosus; SLICC, Systemic Lupus International Collaborative Centers; NPSLE, neuropsychiatric SLE.
[a]Published before the full publication of the EULAR/ACR criteria.
[b]ANA directly tested (not 'ever positive' as per EULAR/ACR criteria).
[c]Positive ANA were an entry criterion for this study.

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with SLICC criteria being met earlier. The LUMINA cohort included patients by ACR criteria. 14% of these patients met the EULAR/ACR and 25% the ACR criteria earlier, with white patients those who met the new criteria later [20]. While the authors reported not being able to include all of the EULAR/ACR criteria variables, it is not clear why their analogue to sensitivity was lower for the EULAR/ACR criteria than in other cohorts.

One additional aspect was disease severity, also termed ominosity [1,2]. Several studies have now shown that those patients whose SLE fulfilled the EULAR/ACR criteria were more prone to severe disease and to damage than those who did not [12,21,22]. While this is outside the legitimate spectrum of classification criteria employment and in contrast with the fact that criteria items accumulate over time [23], Teng *et al.* [24] have even argued that the criteria were reflective of disease activity.

EARLY SYSTEMIC LUPUS ERYTHEMATOSUS

Better performance in classifying early SLE was also a goal of the EULAR/ACR classification criteria project [1,2], which also led to the early SLE cohort study [25]. The EULAR/ACR validation cohort subanalysis on the patients in the early years of their disease suggest that this goal was met [7▪]. In the first year of SLE, the ACR 1997 criteria had 56% sensitivity, as compared with 89% of both the EULAR/ACR 2019 and the SLICC criteria, with a constant level specificity of 92% for each. In the second and third year of disease, ACR criteria sensitivity increased to 81%, but EULAR/ACR criteria sensitivity to 89% and SLICC criteria sensitivity to 87%. Specificity was 95% for the ACR and 96% for the EULAR/ACR criteria, as compared with 88% for the SLICC criteria [7▪]. Similarly, at the time of physician diagnosis of SLE, Adamichou *et al.* [8] found 69% sensitivity for the ACR 1997 criteria, 75% sensitivity for the EULAR/ACR 2019 criteria and 74% sensitivity for the SLICC criteria. The respective specificities were 96% for the ACR, 98% for the EULAR/ACR, and 97% for the SLICC criteria.

PEDIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

In the early phases of the EULAR/ACR criteria project, pediatricians were actively involved [26]. Half of the pediatricians favored one set of criteria for adult and pediatric SLE. Data on pediatric and juvenile SLE patients, that is, those who developed SLE before their 19th birthday, are of particular relevance. The available data [15▪,16▪,17] show improved sensitivity of the EULAR/ACR criteria as compared with the ACR criteria, with the two studies published after the full publication of the EULAR/ACR criteria showing maintained specificity (Table 1).

ANTINUCLEAR ANTIBODIES AS AN OBLIGATORY ENTRY CRITERION

One of the novel features of the new criteria was the repositioning of ANA as an obligatory entry criterion. To reiterate the rationale beyond specificity: there were essentially three options to deal with ANA. To keep them as a specific criterion appeared questionable, given the dismal specificity of the test. To leave them completely out would have discarded an important clinical test with implications for teaching and training. The third possibility was to change their position to the equivalent of the screening test ANA constitute in clinical practice. This was supported by a majority of expert in the Delphi exercise who felt at least not completely comfortable with diagnosing SLE in patients who were always ANA negative [26]. Meta-regression analysis of a systematic literature review concluded 97.8% of SLE patients were positive for ANA at a titer of 1 : 80 or higher, with the lower limit of the 95% confidence interval at 96.8% [27]. The decision to reposition ANA as an entry criterion was also supported by international SLE expert consensus [28].

Nevertheless, the argument was brought forward that ANA as an obligatory entry criterion would exclude a small but relevant proportion of patients. This would obviously be a real issue for diagnosis, but it is important to reiterate that classification criteria should not be used as a diagnostic tool, and certainly not abused for withholding treatment from patients [6]. In contrast, for classification, exclusion of patients would only become an issue if the group was large or would systematically impair the study of relevant subgroups. In the EULAR/ACR criteria cohorts, the percentage of ANA positive patients was always above 99%. The external data so far support sufficient ANA sensitivity. In fact, all the cohorts recently studied for the comparison of the criteria sets showed 94–100% of patients ANA positive (Table 1). Moreover, at the time of diagnosis, 97% of 115 Sudanese SLE patients were ANA positive [29]. Importantly, this also applied to pediatric and juvenile SLE and to early disease. An analysis of SLE patients negative for ANA at a titer of 1 : 160 or higher on HEp-2 cells, in the SLICC inception cohort was 6.2% [30].

Frodlund *et al.* [31] found that 13% of their patients lost ANA positivity over time, which reiterates the importance of ANA ever positive as an entry criterion in the EULAR/ACR criteria. The more

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critical issue was demonstrated by Pisetsky *et al.* [32], who found troublesome differences in the performance of various HEp-2 cell substrates commercially available for ANA testing. Although this is mitigated by the rule that ever positive ANA count, suboptimal ANA testing is a serious issue, and, both on the EULAR and the ACR side, autoimmune serology groups are working to resolve this.

MUCOCUTANEOUS MANIFESTATIONS

Based on interactions between items within the mucocutaneous domain [33▪] and a focus on more prevalent SLE manifestations [34], the mucocutaneous domain changed significantly between the ACR, SLICC and the EULAR/ACR 2019 classification criteria (Table 2).

Grouping in domains results in counting only the highest weighted mucocutaneous item, with weights ranging from 2 for oral ulcers or nonscarring alopecia to 6, more than half of the weight needed for classification at at least 10 points, for malar rash or generalized maculopapular lupus rash. In addition, the domain list became relatively short.

Tarazi *et al.* [35] found that among cutaneous lupus erythematosus (CLE) patients with additional organ involvement beyond mucocutaneous manifestations, a subgroup had negative ANA. Of their 301 CLE patients, 111 were reported always ANA negative, of whom 12 met ACR criteria only and 8 both ACR and SLICC criteria. Their own expert diagnosis of SLE or not SLE was not reported. Of interest, 9 of the ANA negative CLE patients were reported anti-dsDNA positive. Based on their data, Tarazi *et al.* [35] cautioned against the use of ANA positivity as a requirement for diagnosing SLE, with which we fully concur, while we believe that ANA positivity as a requirement for classifying SLE is appropriate.

Zapata and Chong [36], who identified 42 of their CLE patients who had lupus erythematosus lesions not (any more) included in the EULAR/ACR criteria. Of these, 17 (40%) met SLICC classification criteria of SLE, but 12 of these 17 also EULAR/ACR criteria. Accordingly, the number of patients not classified would be small.

Stec-Polak *et al.* [37] positively remarked the higher specificity of the EULAR/ACR 2019 classification criteria. In their cohort of 109 patients with subacute CLE and 75 with discoid lupus erythematosus, the ACR 1997 (same as 1982 in this regard) criteria classified 23%, the SLICC 2012 criteria 17% and the EULAR/ACR 2019 criteria 15% of patients as having SLE. Those patients fulfilling the EULAR/ACR criteria had no severe internal organ involvement.

NEUROPSYCHIATRIC MANIFESTATIONS

While the neurologic manifestations were always grouped together, in the ACR as well as in the SLICC criteria, the SLICC group had expanded the list [3]. The EULAR/ACR criteria project kept the clinically relevant distinction between delirium (acute confusional state in the SLICC criteria), which is

Table 2. Changes in the mucocutaneous criteria items from the American College of Rheumatology to the Systemic Lupus International Collaborative Centers and the European League Against Rheumatism/American College of Rheumatology criteria

ACR criteria	SLICC criteria	EULAR/ACR 2019 criteria
Malar rash	ACLE or SCLE Malar rash Bullous lupus Toxic epidermal necrolysis variant Maculopapular lupus rash Photosensitive lupus rash SCLE	Mucocutaneous domain Malar rash or maculopapular lupus rash (6)
Discoid rash	CCLE Discoid rash Hypertrophic lupus Lupus panniculitis Mucosal lupus LE tumidus Chillblains lupus Discoid lupus/lichen planus overlap	SCLE or DLE (4)
Oral ulcers	Oral or nasal ulcers	Oral ulcers (2)
Photosensitivity	Nonscarring alopecia	Nonscarring alopecia (2)

By grouping into domains, only one (the highest ranking, with relative weights shown in brackets) item can be counted for the EULAR/ACR criteria. ACR, American College of Rheumatology; EULAR/ACR, European League Against Rheumatism/American College of Rheumatology; SLICC, Systemic Lupus International Collaborative Centers. LE, lupus erythematosus; ACLE, acute cutaneous LE; SCLE, subacute cutaneous LE; CCLE, chronic cutaneous LE; DLE, discoid LE.

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Table 3. Changes in the neuropsychiatric criteria items from the American College of Rheumatology to the Systemic Lupus International Collaborative Centers and the European League Against Rheumatism/American College of Rheumatology criteria

ACR criteria	SLICC criteria	Patients [18]	EULAR/ACR 2019 criteria
Neurologic disorder	Neurologic		Neuropsychiatric
Seizures	Seizures	$n=11$ (3.7%)	Seizure (5)
Psychosis	Psychosis	$n=7$ (2.4%)	Psychosis (3)
	Acute confusional state	$n=6$ (2.0%)	Delirium (2)
	Mononeuritis multiplex	$n=0$	
	Myelitis	$n=6$ (2.0%)	
	Neuropathy	$n=3$ (1.0%)	

The numbers and percentages in column 3 refer to patients with these manifestations in the article by Gegenava *et al.* [18], numbers in bracket in column 4 to the relative weights in the EULAR/ACR 2019 criteria. ACR, American College of Rheumatology; EULAR/ACR, European League Against Rheumatism/American College of Rheumatology; SLICC, Systemic Lupus International Collaborative Centers.

characterized by rapid onset and fluctuation throughout the day, and psychosis. Otherwise, however, the items are the same as in the ACR criteria (Table 3).

Gegenava *et al.* [18] investigated the new criteria in their cohort of 294 SLE patients with neuropsychiatric symptoms, which included also cerebrovascular disease ($n=30$), headache ($n=12$), cognitive dysfunction ($n=31$), and mood disorder ($n=9$). The numbers of patients with criteria manifestations are included in Table 3. The EULAR/ACR criteria would not classify nine of 33 patients (27%) with SLICC criteria manifestations, or 3% of this specialized neuropsychiatric cohort, namely six with myelopathy and three with polyneuropathy. For comparison, in the EULAR/ACR cohort, delirium and psychosis showed a prevalence of 0.4 and 1.8%, respectively (Aringer *et al.* article in preparation), half of the Dutch neuropsychiatric SLE cohort. Since items were only included into the EULAR/ACR criteria at an expected sensitivity of at least 1% to keep the criteria list short, noninclusion of these items was probably correct.

CONCLUSION

The EULAR/ACR 2019 classification criteria for SLE have been independently tested by a number of groups worldwide. When applied correctly, the new criteria have good sensitivity and specificity. The external validation data suggest that the EULAR/ACR criteria also perform across subsets of SLE patients including early disease and in pediatric and juvenile SLE.

Acknowledgements

We would like to thank all colleagues who were involved in the SLE classification criteria process. Insights and interpretations in part stem from the discussion in the steering committee, nominal group technique and 1000Minds exercise as well as the Delphi exercise, cohorts studies and writing process.

Financial support and sponsorship

The EULAR/ACR classification criteria project was jointly funded by EULAR and the ACR.

The development of the SLE criteria was jointly funded by EULAR and ACR.

Conflicts of interest

Other than having served as co-chairs of the EULAR/ACR classification criteria steering committee, M.A. and S.R.J. have no conflicts of interest with regard to this article.

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The latest in systemic lupus erythematosus-accelerated atherosclerosis: related mechanisms inform assessment and therapy

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Purpose of Review
Accelerated atherosclerosis is a significant comorbidity and the leading cause of death for patients with systemic lupus erythematosus (SLE). It is now apparent that SLE-accelerated atherosclerosis is not driven solely by traditional cardiovascular risk factors, adding complexity to disease characterization and mechanistic understanding. In this review, we will summarize new insights into SLE-accelerated atherosclerosis evaluation, treatment, and mechanism.

Recent findings
Recent work highlights the need to incorporate inflammatory biomarkers into cardiovascular disease (CVD) risk assessments. This is especially true for SLE patients, in which mechanisms of immune dysfunction likely drive CVD progression. There is new evidence that commonly prescribed SLE therapeutics hinder atherosclerosis development. This effect is achieved both by reducing SLE-associated inflammation and by directly improving measures of atherosclerosis, emphasizing the interconnected mechanisms of the two conditions.

Summary
SLE-accelerated atherosclerosis is most likely the consequence of chronic autoimmune inflammation. Therefore, diligent management of atherosclerosis requires assessment of SLE disease activity as well as traditional cardiovascular risk factors. This supports why many of the therapeutics classically used to control SLE also modulate atherosclerosis development. Greater understanding of the mechanisms underlying this condition will allow for the development of more targeted therapeutics and improved outcomes for SLE patients.

Keywords
atherosclerosis, cardiovascular disease, risk assessment, SLE-accelerated atherosclerosis, systemic lupus erythematosus

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease affecting over 1.5 million Americans and at least 5 million individuals worldwide. Patients are predominantly female, and are two to three times more likely to be women of color. SLE is characterized by autoantibody producing B cells and dysfunctional $CD4^+$ T cells, and can result in end organ damage to the kidneys, joints, and cardiovascular system [1]. Life expectancy for SLE patients has improved but remains lower than that of the general population [2], and the leading cause of death among SLE patients is cardiovascular disease (CVD) driven by accelerated atherosclerosis [3–7]. It is estimated that women with SLE between the ages of 35 and 44 years have a 50-fold increased risk of myocardial infarction compared with age- and sex-matched controls [8]. Even if we assume that this estimate is inflated due to the fact that healthy, premenopausal women are typically protected from CVD development [9], postmenopausal women with SLE are still five times more likely to develop atherosclerosis than healthy, age-matched controls [10]. Statin therapy has been shown to provide less cardiovascular protection in

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Curr Opin Rheumatol 2021, 33:211–218
DOI:10.1097/BOR.0000000000000773

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KEY POINTS

- Accelerated atherosclerosis is the leading cause of death among systemic lupus erythematosus (SLE) patients.
- Cardiovascular disease risk assessment in SLE patients must integrate inflammatory as well as traditional cardiovascular risk factors in order to get an accurate assessment.
- Many of the immune mechanisms that drive SLE also promote atherosclerosis.
- Many of the existing SLE treatments hold promise for improving SLE-accelerated atherosclerosis.

SLE patients compared to the general population [11], suggesting the SLE-accelerated atherosclerosis is not entirely lipid dependent. In this review, we highlight recent findings regarding mechanisms driving SLE-accelerated atherosclerosis as well as the best techniques to evaluate and treat this disease.

DISEASE RISK ASSESSMENT

Atherosclerosis is associated with a number of risk factors and the likelihood of a related adverse event (coronary heart disease, stroke, peripheral artery disease, or heart failure) occurring in the long term in the general population is often determined using composite measurements such as the Framingham Risk Score [12] or Systematic Coronary Risk Evaluation (SCORE) [13]. The Framingham Risk Score is a 10-year predictive measurement that uses sex, age, total and high-density lipoprotein (HDL) cholesterol levels, systolic blood pressure, hypertension, smoking, and diabetes status to determine an individual's CVD risk [12]. SCORE utilizes many of the same metrics, but also incorporates geography as a variable to consider whether an individual's country as a whole has rising or falling CVD rates [13]. In SLE patients, these scores may inaccurately predict an individual's true risk of CVD [14,15]. This is likely due to several factors; the lack of consideration of inflammatory variables that are increased in SLE, the heterogeneity of the disease, and our incomplete understanding of disease mechanism. Therefore, improved methods for predicting risk in SLE are required to ensure appropriate management of CVD and other life-threatening comorbidities.

Techniques for improved risk assessment

One strategy for adjusting the Framingham risk score in SLE has been to simply multiply the traditional score by a factor of two [16]. A similar technique was successfully applied to rheumatoid arthritis patients, who also experience increased rates of CVD related death [17,18]. Unfortunately, given the heterogeneous nature of SLE, a blanket correction factor may oversimplify the contribution of autoimmune factors to CVD risk. Therefore, it became necessary to develop an SLE-centered cardiovascular risk score that uses traditional cardiovascular risk factors like age, gender, systolic blood pressure, cholesterol, smoking, and diabetes, but also SLE-specific risk factors. Variables such as time since SLE diagnosis, corticosteroid and hydroxychloroquine use, C3 and C4 levels, anti-double stranded DNA (dsDNA) titer, proteinuria, disease activity score (SELENA-SLEDAI score), estimated glomerular filtration rate, and history of lupus anticoagulant and anticardiolipin were evaluated. In the subjects of this study, only disease activity score, C3 level, and lupus anticoagulant titer were predictive of cardiovascular outcomes [19▪▪]. Using the SLE-specific risk score incorporating these select variables, Petri *et al.* determined that some SLE patients were appropriately assessed by the traditional Framingham risk score, whereas others, particularly those with higher SLEDAI scores, had their 10-year risk underestimated by as much as a factor of 10 [19▪▪]. In agreement with these findings, a separate 7-year surveillance study found no difference in the progression of subclinical atherosclerosis between the control group and SLE patients with mild, well-controlled disease [20], highlighting the need to use SLE-specific criteria and disease activity level in CVD risk assessment.

Inaccurate CVD risk assessment is especially prevalent in young SLE patients (≤45 years). These patients are not likely to experience adverse cardiovascular events within 10 years (the range in which classical scoring methods are designed to predict), but still have elevated risk compared to their healthy, age-matched counterparts, including a 50-fold increase in the risk of myocardial infarction [8,21]. Clinical studies have shown that coronary artery calcification (CAC), a known predictor of CVD mortality [22], is increased in SLE patients anywhere from 2.8 to 4 times compared to controls and may be an accurate predictor of outcomes [23,24]. These studies were performed using older, mostly white cohorts (72% and 65%, respectively), perhaps not accurately representing the predominately nonwhite SLE-patient population. Gartshteyn *et al.* studied SLE patients aged 18 to 65 years, who were primarily African American and Hispanic, and had no clinical CVD. They found that CAC severity increased with age in patients, and that CAC was significantly more prevalent in young SLE patients compared to age-

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matched controls, 32% versus 9.6%, respectively [25]. In a separate study, a related, although independent condition, aorta calcification (AC), was found to occur earlier, at a higher incidence, and in a wider range of ages than CAC in SLE patients highlighting its importance in predicting atherosclerosis development [26].

Collectively, studies indicate risk assessment for SLE-accelerated atherosclerosis needs to be distinct from that of standard atherosclerosis. By integrating more inflammatory variables into predictive equations and/or adopting regular monitoring of CAC and AC, SLE patients will experience improved care with more timely therapeutic interventions.

Biomarkers

In addition to improved techniques to assess atherosclerosis development in SLE patients, there has also been progress in terms of new biomarkers to identify potentially high CVD risk patients. Biomarkers associated with SLE-accelerated atherosclerosis include proinflammatory HDL (piHDL), increased circulating leptin, elevated homocysteine, and the presence of soluble tumor necrosis factor-like weak inducer of apoptosis (sTWEAK) [27].

Soluble CD163

A biomarker that was previously associated with increased glomerular inflammation in SLE patients is the soluble form of the scavenger receptor CD163 (sCD163). sCD163 was found at high levels in both serum and urine of SLE patients with more $CD163^+$ macrophage glomerular infiltrate and severe inflammation ($r = 0.635$) as part of lupus nephritis [28]. CD163 expression on macrophages has typically been thought of as an indication of alternative activation and an anti-inflammatory phenotype [29]. However, recent findings demonstrated $CD163^+$ macrophages actually promote atherosclerotic plaque progression in both humans and mice via a CD163/HIF1α/VEGF-A pathway [30]. The involvement of $CD163^+$ macrophages in both SLE and atherosclerosis suggest expression of this scavenger receptor may be an underlying mechanism of SLE-accelerated atherosclerosis and that its soluble form could be a biomarker of the disease. This was tested in one study of 63 SLE patients whose carotid atherosclerotic plaque was measured by ultrasound and serum sCD163 levels monitored both at baseline of the study and during follow-up. Results indicated that serum sCD163 is elevated in SLE patients compared to controls, and is further increased in patients with carotid plaque. Additionally, higher levels of sCD163 correlated with the development of new carotid plaque over time and positively associated with subclinical atherosclerosis in SLE independent of classic cardiovascular risk factors [31▪]. These results indicate sCD163 is a promising biomarker of SLE-accelerated atherosclerosis.

FcγRIIA polymorphism

Fcγ receptors (FcγRs) are expressed on antigen presenting cells, such as macrophages, dendritic cells (DCs), and B cells, and bind to the Fc domain of IgG antibodies. Depending on whether the FcγR is activating or inhibitory, it will promote pro- or anti-inflammatory responses [32]. In mouse models, activating FcγRs are proatherogenic [33]. Functional polymorphism in the activating Fcγ receptor IIA (FcγRIIA) have previously been associated with SLE and lupus nephritis in humans [34,35]. In heparin-induced thrombocytopenia, a functional polymorphism (single amino acid substitution H131R) in FcγRIIA caused a hyperactive platelet phenotype characterized by increased expression of P-selectin and CD40 ligand, greater binding to annexin V, and increased formation of platelet-leukocyte complexes [36–40]. A similar phenotype was described in SLE where hyperactive platelets promote vascular pathogenesis by activating endothelial cells [41]. Based on these works, Clancy *et al.* investigated whether the H131R FcγRIIA polymorphism impacted cardiovascular outcomes in SLE patients. The H131R allelic variant was found in a similar proportion (~40%) of both the SLE patient and healthy control populations. When SLE patients were assessed by carotid ultrasound, 58% of SLE patients with the allelic variant had carotid plaque compared to only 25% of SLE patients with the ancestral variant. Yet, the FcγRIIA allelic variant did not associate with carotid plaque in heathy control subjects [42▪]. This suggests the H131R functional polymorphism confers CVD risk specifically in the context of SLE and could potentially act as a genetic CVD biomarker in patients.

Appropriate CVD risk assessment for the SLE patient population has long been needed. The techniques and biomarkers outlined above have the potential to improve CVD risk detection in patients, hopefully resulting in prompt therapeutic intervention and prevention of devastating cardiovascular outcomes.

MECHANISM AND TREATMENT

Treatments for SLE are limited in comparison to other chronic illnesses and therapeutic advancement has been relatively slow, in part because of lack of understanding of disease mechanisms. Unfortunately, the impact of some of the most common SLE therapeutics specifically on SLE-accelerated atherosclerosis has been understudied. The

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Table 1. Review of SLE-accelerated atherosclerosis treatments

Treatment	Effect in SLE-accelerated atherosclerosis	References
BAFF inhibition	Improves SLE responder index in patients with ongoing B cell dysfunction Reduces circulating anti-dsDNA antibodies in mice Inhibits antibody and complement deposition in the kidney in mice Atheroprotective in animals with low (<5mmol/L) cholesterol and atherogenic in animals with high cholesterol	[52,54▪▪]
Hydroxychloroquine	Reduces interferon-α production Lessens aortic stiffness in SLE patients Corrects lipoprotein profile (increases HDL and lowers cholesterol, LDL, VLDL, triglycerides, and cylomicrons) in SLE patients Improves glycemic control in nondiabetic women with SLE Reduces the risk of all thrombovascular events in SLE patients SLE patients with carotid plaque are less likely to be using HCQ than those with no plaque (63% vs. 82.3%) Long term HCQ treatment in *ApoE*$^{-/-}$ mice with pristane-induced SLE reduces atherosclerotic lesion size, anti-dsDNA antibodies, total leukocyte numbers, macrophages, and DCs, and increases lymohocytes HDL from SLE patients is more functional following 12 weeks of treatment with HCQ.	[58–66,67▪,70▪]
Low-dose IL-2	IL-2 treatment reduces renal inflammation and activation of kidney-infiltrating CD4$^+$ T cells in a mouse model of lupus nephritis Targeted IL-2 treatment in *ApoE*$^{-/-}$ mice reduces the size of pre-established atherosclerotic lesions Currently under study in clinical trials to determine its effectiveness in SLE and atherosclerosis	[96▪,97–99]
Mycophenolate	Treatment with MMF for 12 weeks improves HDL function in SLE patients Treatment with MMF for 12 weeks improves CVD biomarkers like sTWEAK in SLE patients Reduces atherosclerosis in *Ldlr*$^{-/-}$ B6.Sle1.2.3 bone marrow chimeras and limits recruitment of CD4$^+$ T cells to atherosclerotic lesions	[70▪,71]

SLE, systemic lupus erythematosus; VLDL, very low-density lipoprotein.

following highlights recent work that has contributed to determining the impact of SLE treatments on atherosclerosis and the mechanism behind SLE-accelerated atherosclerosis. Table 1 summarizes the treatments discussed and their effects.

In SLE, B cells are hyperactive leading to the production of autoantibodies, inappropriate T cell activation and DC recruitment, and inhibition of regulatory T cells (T_{regs}) [43]. In the context of atherosclerosis, B cells were initially thought to be protective [44,45], but recent work has demonstrated B2 B cells and production of IgG are proatherogenic [46,47]. The cytokine B-cell activating factor (BAFF) is required for the maturation and survival of B2 B cells and plays a major role in SLE [48–51]. Benlysta, a B-cell-depleting monoclonal antibody that targets BAFF, was the first new SLE therapy in 50 years [52]. Therefore, inhibition of BAFF signaling seemed an attractive target for treating SLE-accelerated atherosclerosis. To test this, Saidoune *et al.* crossed atherosclerosis-susceptible *ApoE*$^{-/-}$ mice to Qa-1 knock-in mice (*D227K*) that have an amino acid mutation that hinders CD8$^+$ T_{regs} and develop an SLE-like phenotype [53]. Interestingly, when *ApoE*$^{-/-}$ *D227K* mice were treated with a BAFF neutralizing antibody, SLE disease activity was impaired but atherosclerosis severity was only improved in animals with low cholesterol levels (<5 mmol/L). This unexpected finding was the result of BAFF signaling through transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) on macrophages. Inhibiting BAFF-TACI signaling on macrophages promoted foam cell formation in high lipid environments and was largely atherogenic, overriding the atheroprotective effect of disrupting BAFF-BAFF receptor (BAFFR) on B cells in SLE [54▪▪]. A separate study demonstrated BAFFR is also expressed on circulating endothelial progenitor cells (EPCs) [55▪], a population of cells that are meant to maintain vascular homeostasis but that is reduced in SLE patients [56,57]. BAFF signaling on EPCs induced apoptosis suggesting inhibition of BAFF signaling could be beneficial in SLE-accelerated atherosclerosis [55▪]. Overall, BAFF signaling seems to be a critical mechanism of SLE-accelerated atherosclerosis, but requires further study due to its complex interaction with multiple cell types.

Hydroxychloroquine (HCQ) is one of the most widely used medications for autoimmune diseases and is regularly prescribed for SLE [58]. It is known to elicit atheroprotective effects like improving lipid profiles [59–61] and glycemic control [62], lessening aortic stiffness [63], and reducing thrombotic events

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[64,65]. There is a documented negative correlation between HCQ treatment and atherosclerosis in SLE [66], but the mechanism has not been elucidated. By studying pristane-induced SLE in atherosclerosis-susceptible *ApoE*$^{-/-}$ mice, Liu *et al.* showed long-term HCQ treatment reduced atherosclerosis largely through reversal of SLE-associated autoinflammation. Specifically, HCQ treatment lowered anti-dsDNA antibodies and reduced total leukocytes, macrophages, and DCs [67▪]. Pristane-induced SLE is known to reduce lymphocyte numbers in the spleen by triggering their apoptosis [68]. This phenotype was also reversed by HCQ treatment in both the aorta and the spleen which authors hypothesize increased the numbers of atheroprotective T_{regs} [67▪]. This mechanistic study provides some insight to how HCQ may also be beneficial in treating SLE-accelerated atherosclerosis.

Although typically considered to be atheroprotective due to its anti-inflammatory properties, HDL in SLE patients can become piHDL. SLE patients with carotid artery plaque are more likely to have piHDL than SLE patients without plaque [69], making it an interesting biomarker for SLE-accelerated atherosclerosis [27]. Little is known about the effects of regularly prescribed SLE disease-modifying therapies like mycophenolate (MMF), azathioprine (AZA), and HCQ on piHDL. In a recent observational study, SLE patients starting a new disease-modifying therapy (MMF, AZA, or HCQ) had plasma collected and disease activity measured at baseline, 6 weeks post initiation of therapy, and 12 weeks post initiation of therapy [70▪]. MMF and HCQ significantly improved HDL function over the course of the 12-week treatment, although not to normal levels, whereas AZA had no effect on HDL function. MMF also significantly improved other biomarkers associated with plaque and intima-media thickness progression in SLE, suggesting MMF could have potential to treat both SLE associated glomerulonephritis and accelerated atherosclerosis [70▪].

Findings from our own work demonstrate that MMF treatment reduces atherosclerosis in *Ldlr*$^{-/-}$ B6.*Sle1.2.3* bone marrow chimeric mice (which spontaneously develop SLE and are atherosclerosis prone) and inhibits CD4$^+$ T cell activation and recruitment to atherosclerotic plaques [71]. In general, T cells are known to significantly contribute to the development of atherosclerosis [72,73], with different subsets playing unique roles. For instance, Th1 cells are widely accepted to be proatherogenic [74–76], whereas functional T_{regs} are atheroprotective [77,78]. T cells are also critical in SLE where they are long-lived, hyperactive, and produce proinflammatory cytokines like IFN-γ and IL-17 [79–82]. Much of the work from our group has shown the importance of T cells in mouse models of SLE-accelerated atherosclerosis [83–85], and our findings are in agreement with results from human studies [86,87].

Previous results from Laurence Morel's group identified the lupus susceptibility gene, *Pbx1d*, as overexpressed in CD4$^+$ T cells from SLE patients and using mouse models of SLE described its role in expanding follicular helper T cells (Tfh) and impairing T_{reg} homeostasis [88,89]. Given the importance of CD4$^+$ T cells in atherosclerosis, recent work explored the effect of *Pbx1d* transgenic CD4$^+$ T cells in atherosclerosis in *Ldlr*$^{-/-}$ bone marrow chimeras. *Pbx1d* overexpression in CD4$^+$ T cells resulted in thicker arterial walls and larger necrotic cores within lesions, which is thought to be an indication of plaque instability and severity. Recipients of *Pbx1d* transgenic bone marrow had expanded Tfh cells and impaired T_{regs}, that was increased with western diet feeding [90▪▪], indicating a lupus susceptibility allele can drive atherosclerosis, while at the same time, dyslipidemia can enhance autoimmune phenotypes.

Impaired IL-2 signaling in T cells due to decreased IL-2 production in SLE and its contribution to disease pathogenesis is well known [91–94]. Recent findings identify a regulatory subunit (PPP2R2D) of protein phosphatase 2A (an enzyme which is increased in T cells from SLE patients and leads to decreased IL-2 production) that limits the accessibility of the *IL-2* gene and other transcription factors important to IL-2 expression via chromatin remodeling. PPP2R2D is increased in T cells from SLE patients and PPP2R2D deficiency in T cells prevents autoimmunity in an imiquimod-induced lupus-like mouse model [95▪]. A separate group demonstrated that treating (NZB × NZW) F1 SLE mice with IL-2 reduced renal inflammation and lessened the activity of kidney-infiltrating CD4$^+$ T cells [96▪]. Interestingly, IL-2 delivered specifically to atherosclerotic lesions through fusion to fibronectin targeting antibody reduced plaque size by activating and expanding T_{regs} [97]. This suggests that IL-2 supplementation could be beneficial in both SLE and atherosclerosis, a hypothesis that is currently being tested in low-dose IL-2 treatment clinical trials [98,99].

In order to determine how the risk of atherosclerotic vascular events (AVE) in SLE patients has changed over time in response to new therapeutic strategies, Urowitz *et al.* compared two cohorts of newly identified (enrolled in the study within 12 months of diagnosis) SLE patients. Cohort 1 included patients who entered the University of Toronto Lupus Clinic between 1975 and 1987 and were then followed through 1992, whereas Cohort 2 included patients entering between 1999 and 2011 and were followed through 2016. Over the course of

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study SLE disease activity, treatment regimens, blood pressure, cholesterol, blood sugar, smoking status, and AVE were monitored. Patients in Cohort 2 had significantly less AVE than Cohort 1 over the 17-year study period. Additionally, patients in Cohort 2 had lower SLE disease burdens, smoked less and had longer periods of time with normal blood pressure, cholesterol, and glucose than patients in Cohort 1, demonstrating interventions for both SLE and CVD have improved over time [100▪].

Understanding how current treatment regimens effect SLE-associated atherosclerosis is an important goal that will provide near immediate benefit to individuals living with the disease. Of equal importance, is the more long-term goal of elucidating the mechanisms that drive SLE-accelerated atherosclerosis. Although findings made in this area may not immediately translate to the clinic, they will eventually lead to more targeted therapeutics for patients. These works suggest a highly interconnected, positive feedback loop between progression of SLE and atherosclerosis, and may provide further insight as to why therapeutics designed to treat one impact the other.

CONCLUSION

SLE-accelerated atherosclerosis is a complex disease in which immunological and cardiovascular mechanisms are intertwined to drive pathogenesis. Although recent work has begun to elucidate this interconnected relationship, further study is needed to gain a complete mechanistic understanding of the disease and to identify potential therapeutic targets. In the meantime, the field has grown in its understanding of how to best evaluate current SLE patients for their cardiovascular risk, integrating traditional cardiovascular risk factors with information about inflammatory disease activity. This improvement in disease risk assessment will lead to better monitoring of high-risk patients as well as improved treatment regimens.

Acknowledgements

None.

Financial support and sponsorship

Support provided by the Veterans Association (VA Merit Award I01BX002968 to A.S.M) and the National Institutes of Health (NIAID R01AI153167 to A.S.M and NHLBI 1F31HL154569-01 to B.D.A).

Conflicts of interest

There are no conflicts of interest.

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