Nature Reviews Rheumatology | Published online 21 Apr 2017; doi:10.1038/nrrheum.2017.41

OSTEOARTHRITIS

Metabolic syndrome and risk of knee OA

Previous studies have suggested that metabolic syndrome is associated with an increased risk of knee osteoarthritis (OA). However, an analysis of data from the Framingham OA Study, a substudy of the Framingham Heart Study, has revealed that neither metabolic syndrome nor its components, with the exception of hypertension, are associated with incident OA after adjustment for BMI or body weight.

Metabolic syndrome and its relationship with heart disease has been a major focus of the Framingham Heart Study. Measurements of metabolic syndrome, along with information about the development of OA, enabled Niu *et al.* to assess the relationship of metabolic syndrome and its individual components (which include central obesity, dyslipidemia, hypertension and impaired fasting glucose) with the risk of knee OA. They were also able to adjust for body weight or obesity — an important consideration given the strong relationship between obesity and occurrence of metabolic syndrome.

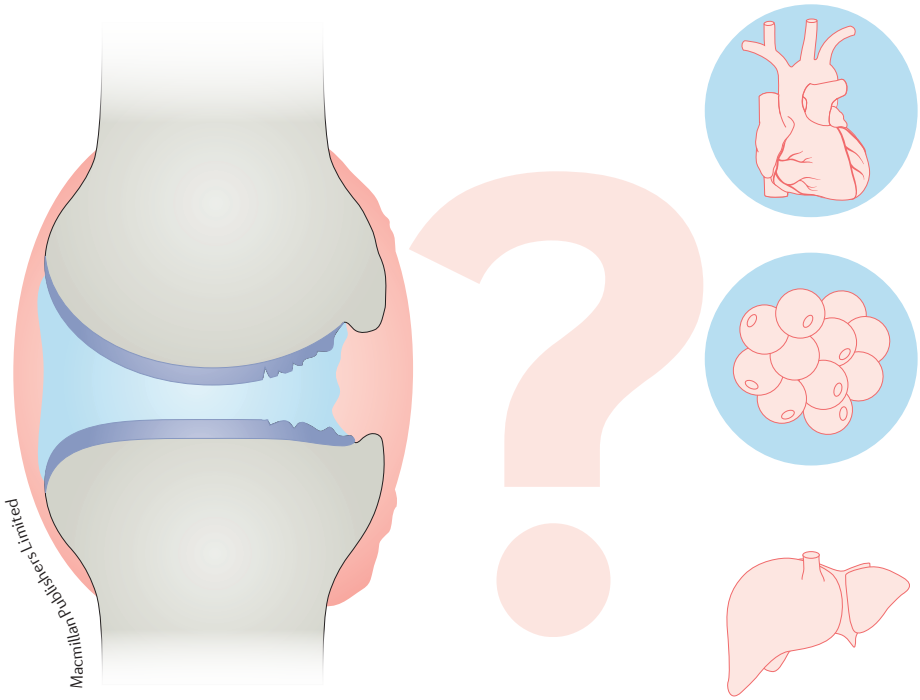
> “…most [associations between metabolic syndrome components and knee OA] were attenuated after adjustment for BMI…”

The longitudinal study analysed data from 991 individuals aged ≥40 years (mean age 54.2 years) without prevalent radiographic knee OA at baseline (1992–1995) who returned for a follow-up assessment 10 years later. Assessment of metabolic syndrome and its components was performed ~1 year before the baseline OA examination. According to US National Cholesterol Education Program Adult Treatment Panel III criteria, 26.7% of men and 22.9% of women in the study had metabolic syndrome, although data was not available to account for changes in metabolic syndrome or its components over the follow-up period.

At follow-up, the incidence of radiographic OA was 9.8% in men and 10.5% in women, and the incidence of symptomatic OA 6.3% in men and 7.2% in women. Preliminary analysis indicated that metabolic syndrome was associated with radiographic OA in men and with symptomatic OA in women, and the presence of more individual components of metabolic syndrome was associated with radiographic and symptomatic OA in both men and women. However, these trends were not statistically significant after adjustment for BMI or body weight.

Individual components of metabolic syndrome, in particular abdominal obesity, were also associated with incident knee OA, but most of these associations were attenuated after adjustment for BMI or body weight. Only hypertension (specifically, increased diastolic blood pressure) remained associated with an increased incidence of symptomatic knee OA in both sexes. Surprisingly, Niu *et al.* found that a high fasting blood glucose level was inversely associated with the incidence of symptomatic knee OA, whereas a positive association had been noted in earlier studies. Hypertension has previously been linked with OA, and Niu *et al.* suggest this association warrants further study.

Sarah Onuora



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Nature Reviews Rheumatology | Published online 2 Mar 2017; doi:10.1038/nrrheum.2017.28

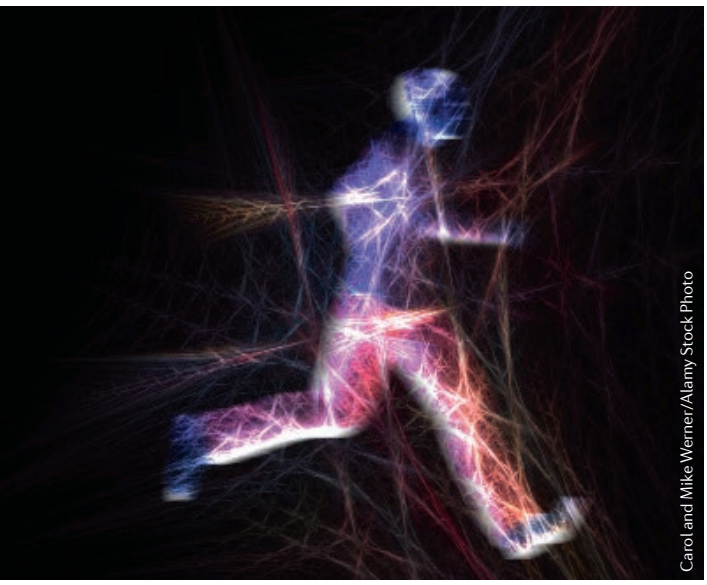
IMMUNOMETABOLISM

IL-37 fights inflammation-induced fatigue

> treatment with IL-37 increased the flux of oxidative phosphorylation substrates and levels of AMPK in skeletal muscle

New research by Giulio Cavalli and colleagues shows that the IL-1 family cytokine IL-37 not only has anti-inflammatory effects, but also induces metabolic reprogramming both in the context of inflammation-induced fatigue and in healthy mice. "We demonstrate a remarkable property of IL-37 to limit the metabolic costs of inflammation and to foster exercise tolerance," explains co-author Charles Dinarello.

In mice with systemic inflammation induced by administration of low-dose lipopolysaccharide (LPS) (an established model of inflammation-induced fatigue associated with reduced exercise tolerance), treatment with recombinant human IL-37 reduced levels of inflammatory markers in muscle and serum and restored endurance running time to near baseline levels. "Given the known suppression of systemic inflammation by IL-37, it was not unexpected that exercise tolerance would improve upon IL-37 treatment," remarks Dinarello. "However, the beneficial effects of IL-37 on exercise tolerance were more marked than those of anakinra, the anti-inflammatory IL-1 receptor antagonist, thus implying additional mechanisms of action beyond suppression of inflammation," he continues.



Carol and Mike Werner/Alamy Stock Photo

Surprisingly, IL-37 treatment also markedly improved exercise tolerance in healthy mice not subjected to LPS-induced inflammation. Endurance running time improved within 24 h of IL-37 administration, and by day 2 was 82% higher in IL-37-treated mice compared with vehicle-treated mice ($P = 0.01$); after eight daily doses of IL-37, the increase was 326% ($P = 0.001$). Cavalli *et al.* also showed that the effects of IL-37 on exercise tolerance are mediated by the IL-1 decoy receptor IL-1R8 (also known as TIR8, or single immunoglobulin IL-1R related receptor) and AMP-activated protein kinase (AMPK), as they were abrogated by AMPK inhibition or IL-1R8 deficiency.

"These effects of IL-37 on exercise tolerance in healthy mice were not secondary to suppression of the inflammatory response," says Dinarello. "Rather, we observed direct and profound effects on energy metabolism." Specifically, treatment with IL-37 increased the flux of oxidative phosphorylation substrates and levels of AMPK in skeletal muscle. "Metabolomics analyses also revealed reduced levels of the proinflammatory mediator succinate and oxidative stress-related metabolites, and changes in amino acid and purine metabolism in muscles of treated animals," adds Dinarello.

Fatigue is a debilitating but often neglected manifestation of chronic inflammatory diseases. "Our study provides the rationale for development of recombinant IL-37 in the treatment of inflammation-induced fatigue," concludes Dinarello. "The ultimate goal is to have recombinant forms of IL-37 available to physicians to treat humans."

Sarah Onuora

ORIGINAL ARTICLE Cavalli, G. *et al.* Interleukin 37 reverses the metabolic cost of inflammation, increases oxidative respiration, and improves exercise tolerance. *Proc. Natl Acad. Sci. USA* http://dx.doi.org/10.1073/pnas.1619011114 (2017)

Nature Reviews Rheumatology | Published online 23 Mar 2017

IN BRIEF

INFECTION

Therapeutics and risk of infection in pregnancy

Data from a large-scale observational study of pregnant women with systemic inflammatory conditions (n = 4,961) shows that the risk of serious infection is similar among those taking steroids (3.4%), non-biologics (2.3%) or TNF inhibitors (1.5%). Comparisons between these groups found no statistically significant differences, giving hazard ratios for non-biologics versus steroids, TNF inhibitors versus steroids, and TNF inhibitors versus non-biologics of 0.81, 0.91 and 1.36, respectively. Dose–response analysis indicated that high steroid doses were independently associated with an increased risk of serious infection in pregnancy.

ORIGINAL ARTICLE Desai, R. J. *et al.* Risk of serious infections associated with use of immunosuppressive agents in pregnant women with autoimmune inflammatory conditions: cohort study. *BMJ* http://dx.doi.org/10.1136/bmj.j895 (2017)

OSTEOARTHRITIS

A new tool for measuring synovial inflammation

Researchers have developed a new method for measuring knee joint effusion-synovitis volume, an MRI marker of synovial inflammation. This new approach gave reproducible volume measurements that were highly correlated with effusion-synovitis scores. In patients with symptomatic knee osteoarthritis who had low baseline levels of vitamin D and effusion-synovitis (n = 413), monthly supplementation with vitamin D_3 (50,000 IU) over 24 months retarded the progression of effusion-synovitis compared with the placebo group (-1.94 ml, 95% CI -3.54 to -0.33).

ORIGINAL ARTICLE Wang, X. *et al.* Knee effusion-synovitis volume measurement and effects of vitamin D supplementation in patients with knee osteoarthritis. *Osteoarthritis Cartilage* http://dx.doi.org/10.1016/j.joca.2017.02.804 (2017)

RISK FACTORS

Generational differences in arthritis prevalence

A longitudinal study of four birth cohorts (1935–1944, n = 1,598; 1945–1954, n = 2,208; 1955–1964, n = 2,781; and 1965–1974, n = 2,230) found that succeeding generations had a higher prevalence of arthritis. Various risk factors were associated with arthritis but did not account for this cohort effect. The benefits of improved education, increased income and smoking cessation on arthritis prevalence partially overcame the detrimental effects of increasing BMI. Further analysis suggested that obese individuals had an earlier age of arthritis onset compared with individuals of normal weight.

ORIGINAL ARTICLE Badley, E. M. *et al.* A population-based study of changes in arthritis prevalence and arthritis risk factors over time: Generational differences and the role of obesity. *Arthritis Care Res. (Hoboken)* http://dx.doi.org/10.1002/acr.23213 (2017)

THERAPY

Monitoring risk of anterior uveitis in AS

In a study of patients with ankylosing spondylitis (AS), the rate of anterior uveitis was reduced upon treatment with adalimumab (n = 406) or infliximab (n = 605) compared with pretreatment rates. The opposite was seen in patients receiving etanercept treatment (n = 354). Treatment with etanercept was associated with an increased risk of anterior uveitis compared with adalimumab (HR 3.86, 95% CI 1.85–8.06) or influximab (HR 1.99, 95% CI 1.23–3.22), whereas no significant differences were observed between adalimumab and infliximab treatment.

ORIGINAL ARTICLE Lie, E. *et al.* Tumour necrosis factor inhibitor treatment and occurrence of anterior uveitis in ankylosing spondylitis: results from the Swedish biologics register. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2016-210931 (2017)

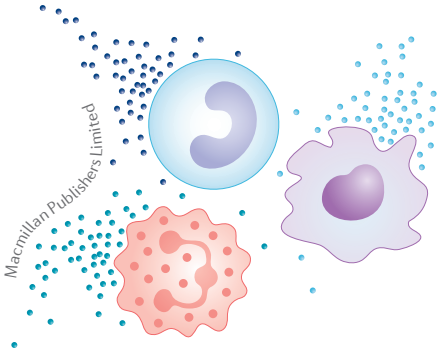
Nature Reviews Rheumatology | Published online 23 Mar 2017; doi:10.1038/nrrheum.2017.44

SYSTEMIC LUPUS ERYTHEMATOSUS

Defective CD11b raises IFN levels in SLE

> Patients carrying *ITGAM* risk alleles had higher serum levels of type I IFN than those who did not...

Genetic variants of *ITGAM* that produce integrin αM (also known as CD11b) with reduced activity are associated with increased levels of type I interferon (IFN) in patients with systemic lupus erythematosus (SLE), according to new research. "Mutations in the coding region of *ITGAM*, which codes for CD11b, strongly associate with an increased risk of SLE," states Vineet Gupta, one of the corresponding authors on the study. "We wanted to identify the molecular mechanism behind how mutant CD11b increases disease risk in SLE and whether it could be therapeutically targeted," he continues.



Gupta and colleagues measured the levels of type I IFN in 171 patients with SLE who were tested for single nucleotide polymorphisms in the coding region of *ITGAM*. Patients carrying *ITGAM* risk alleles had higher serum levels of type I IFN than those who did not carry *ITGAM* risk alleles, although levels of disease activity were not significantly different between the two groups.

The researchers used a genome-wide mRNA screening approach to identify components of Toll-like receptor (TLR) signalling pathways involved in CD11b-mediated type I IFN production. "Our mechanistic studies *in vitro* suggest that CD11b negatively regulates the TLR-dependent AKT–FOXO3–IRF3/7 pathway to control type I IFN production and that this pathway is dysregulated in patients carrying *ITGAM* risk alleles," says Gupta.

To investigate whether defective CD11b activity can be rescued therapeutically, the research team utilized a small-molecule agonist of CD11b called LA1 that they had discovered previously. LA1 treatment activated integrins and reduced the production of type I IFN in wild-type mice but not in mice lacking CD11b. *In vitro*, LA1 activated mutant CD11b and suppressed TLR signalling with equal efficacy to wild-type CD11b. *In vivo*, treatment with LA1 ameliorated disease in the MRL/lpr mouse model of lupus, with reduced renal, skin and cardiovascular manifestations in mice treated with LA1 compared with vehicle-treated mice.

"Altogether, these data suggest that LA1 can activate both wild-type and mutant CD11b and that such CD11b activation is able to modulate or control TLR signalling in cells," explains Gupta. "We now have a novel small molecule that can perhaps be moved forward as a therapeutic lead for SLE as well as lupus nephritis clinical trials," he concludes.

Joanna Collison

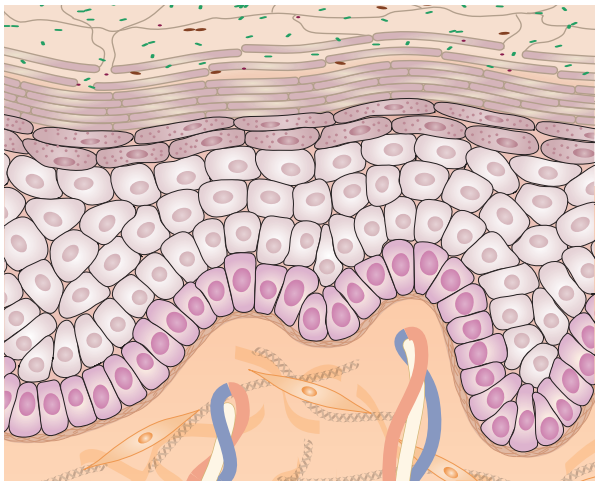
ORIGINAL ARTICLE Faridi, M. H. *et al.* CD11b activation suppresses TLR-dependent inflammation and autoimmunity in systemic lupus erythematosus. *J. Clin. Invest.* http://dx.doi.org/10.1172/JCI88442 (2017)

Nature Reviews Rheumatology | Published online 16 Mar 2017; doi:10.1038/nrrheum.2017.39

SYSTEMIC SCLEROSIS

Knocking out FLI1 to clear the AIRE

A new mouse model has shed light on the autoimmune phenotype observed in systemic sclerosis (SSc) and the unexplained tissue specificity of this disease. In this mouse model, conditional deletion of the transcription factor FLI1 in epithelial cells recapitulates the disease phenotype observed in patients with SSc. "Our findings seemed to prove the notion that 'global' epithelial abnormality plays a fundamental role in this disease," reports corresponding author Yoshihide Asano.



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Numerous studies have previously implicated FLI1 in SSc pathogenesis. Asano *et al.* observe that FLI1 expression is lower in keratinocytes from patients with SSc than in those from healthy individuals, and that gene silencing of FLI1 in keratinocytes from healthy individuals results in an SSc-like gene expression profile. To further investigate the role of FLI1 in murine keratinocytes, the researchers crossed *K14-Cre* mice with *Fli1*$^{fl/fl}$ mice to generate mice in which FLI1 is knocked out in keratinocytes, some oesophageal epithelial cells and medullary thymic epithelial cells (mTECs), referred to here as *K14-Cre.Fli1*$^{fl/fl}$ mice. "These mice exhibited an unexpectedly robust phenotype of autoimmunity as well as skin and oesophageal fibrosis. These phenotypes closely resemble human SSc phenotypes in various aspects," explains Asano.

The autoimmune phenotype in *K14-Cre.Fli1*$^{fl/fl}$ mice included spontaneous development of interstitial lung disease (ILD). Using an adoptive T-cell transfer system, Asano and colleagues showed that the development of ILD, but not skin and oesophageal fibrosis, in *K14-Cre.Fli1*$^{fl/fl}$ mice was dependent on autoreactive T cells. The researchers hypothesized that loss of FLI1 in mTECs could impair the expression of autoimmune regulator (AIRE). After confirming that murine mTECs strongly express FLI1 and that mTECs lacking FLI1 show reduced AIRE expression, Asano and colleagues used human keratinocytes to verify that FLI1 directly regulates *AIRE* transcription.

These results highlight the important role of FLI1 in autoimmune regulation, marking it out as a potentially therapeutic candidate. "Development of gene-modifying drugs that upregulate FLI1 expression might have beneficial effects in the treatment of patients," proposes Asano. "As skin keratinocytes and the epidermis are easily accessible by topical treatment this new approach might be advantageous."

Jessica McHugh

ORIGINAL ARTICLE Takahashi, T. *et al.* Epithelial Fli1 deficiency drives systemic autoimmunity and fibrosis: Possible roles in scleroderma. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20160247 (2017)

Nature Reviews Rheumatology | Published online 6 Apr 2017; doi:10.1038/nrrheum.2017.55

EXPERIMENTAL ARTHRITIS

IL-38 promotes anti-inflammatory effects

A new study shows that IL-38 — a member of the IL-1 cytokine family — reduces inflammation in two experimental models of inflammatory arthritis and promotes an anti-inflammatory effect in macrophages and fibroblasts. "IL-38 seems to be a broad anti-inflammatory cytokine and might not be an antagonist of a specific pathway as previously suggested," says Benoit Le Goff, corresponding author on the paper.

Previous work by this and other groups demonstrated that IL-38 expression is upregulated in patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), and that polymorphisms in the gene encoding IL-38 are associated with ankylosing spondylitis and RA. Furthermore, studies in mice indicated that IL-38 is a negative regulator of inflammatory arthritis.

To investigate the anti-inflammatory function of IL-38, Le Goff and colleagues injected adeno-associated viruses encoding the immature form of IL-38 into the joints of mice in several models of arthritis, including collagen-induced arthritis (CIA), the K/B×N serum transfer model and antigen-induced arthritis (AIA). "In contrast to other IL-1 family members, the mature form of IL-38 has not been characterized and active recombinant IL-38 is not available," explains Le Goff. Compared with control mice treated with GFP, IL-38 overexpression significantly reduced clinical inflammation in mice with CIA and K/B×N mice during the peak and resolution phases of arthritis, whereas no difference was observed in mice with AIA at any time point. IL-38 overexpression in mice with CIA was associated with a reduced number of macrophages in the inflamed synovial tissue and significantly reduced gene expression level of cytokines such as IL-17, IL-22 and IL-23.

To confirm its role in cytokine production, the investigators overexpressed IL-38 in the THP1 monocytic cell line. Protein levels of IL-6, IL-10, IL-23 and TNF were reduced in lipopolysaccharide (LPS)-stimulated THP1 cells after IL-38 overexpression compared with LPS-stimulated control THP1 cells. Moreover, compared with conditioned media from THP1 control cells, conditioned media from IL-38-overexpressing THP1 cells — which was found to contain IL-38 — reduced the secretion of IL-6 and IL-23 in LPS-stimulated M1 macrophages from healthy donors and reduced the production of IL-6 in IL-1β-stimulated fibroblast-like synoviocytes from patients with RA.

> "IL-38 overexpression significantly reduced clinical inflammation in [mouse models of arthritis]"

These findings indicate that IL-38 attenuates the severity of arthritis by reducing the number of macrophages and the expression of proinflammatory cytokines. "We need now to identify the mature form of IL-38 and the receptor used by this cytokine to dampen inflammation," remarks Frédéric Blanchard, co-corresponding author on the paper. "We also need to better understand the role of IL-38 in cartilage and bone loss because prevention of structural damages represents a major clinical need in inflammatory arthritis," Blanchard concludes.

Dario Ummarino

ORIGINAL ARTICLE Boutet, M-A. *et al.* IL-38 overexpression induces anti-inflammatory effects in mice arthritis models and in human macrophages *in vitro*. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2016-210630 (2017)

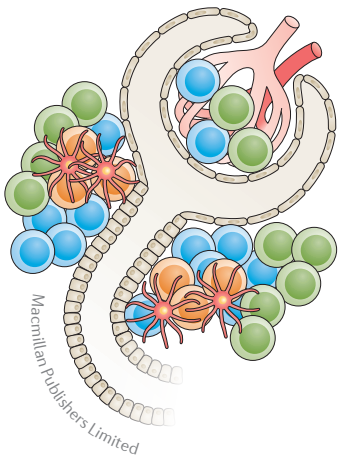
Nature Reviews Rheumatology | Published online 16 Mar 2017; doi:10.1038/nrrheum.2017.40

LUPUS NEPHRITIS

Novel role for BAFF in tertiary lymphoid neogenesis

> "reducing BAFF levels … caused T cells in the kidney to be positioned outside of the glomeruli"

Research published in *The Journal of Immunology* reveals an unexpected role for B cell activating factor (BAFF, also known as TNF super family member 13B) in the formation of tertiary lymphoid structures (TLSs) in the kidneys of lupus-prone mice. "Although the presence of TLSs in non-lymphoid organs such as the kidneys has been reported, what causes their formation has remained unknown," explains Barbara Vilen, corresponding author on the study. "We defined a novel role for BAFF in the formation of TLSs and in the positioning of T cells during lupus nephritis," she states.



BAFF levels are raised in patients with systemic lupus erythematosus and anti-BAFF therapy has shown promise in clinical trials. "Past studies mainly implicated BAFF in breaking B cell tolerance by allowing autoreactive B cells to compete for follicular niches, but several things remained unknown," says Vilen. "First, what causes increased BAFF production during disease, and second, does BAFF play a role in lupus nephritis?"

After passive transfer of anti-nucleosome antibodies into lupus-prone AID$^{-/-}$ MRL/lpr mice (which lack IgG and do not spontaneously develop lupus nephritis), Vilen and colleagues saw an increase in the migration of immune cells into the kidneys, which was coordinated by the binding of immune complexes to Fcγ receptors. The number of TLSs in the kidneys increased sixfold, and the number of BAFF-producing cells in the kidneys increased threefold in mice injected with anti-nucleosome antibodies as compared with those injected with saline.

Reducing BAFF levels by administration of soluble BAFF receptor to lupus-prone mice reduced serum levels of autoantibodies, prevented the development of renal TLSs and limited nephritis, although it did not reduce the infiltration of immune cells into the kidney. Intriguingly, reducing BAFF levels in these mice caused T cells in the kidney to be positioned outside of the glomeruli. "How BAFF does this remains unclear since BAFF is not a chemoattractant," states Vilen. "However, taken together, our study mechanistically links immune complexes, Fcγ receptors and BAFF in lupus nephritis, and in the formation of renal TLSs," she concludes.

Joanna Collison

ORIGINAL ARTICLE Kang, S. *et al.* BAFF induces tertiary lymphoid structures and positions T cells within the glomeruli during lupus nephritis. *J. Immunol.* http://dx.doi.org/10.4049/jimmunol.1600281 (2017)
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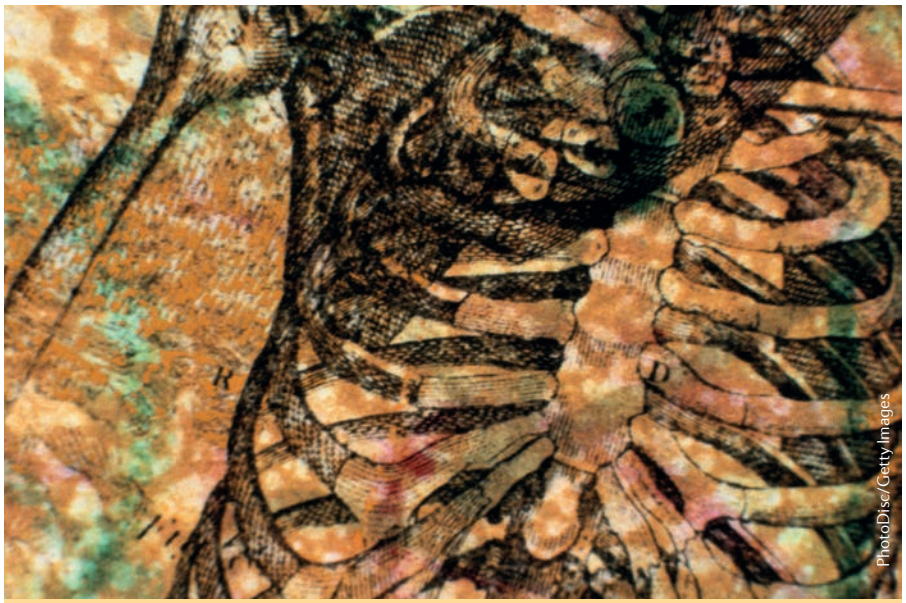

BONE

Osteoblasts and global energy metabolism – beyond osteocalcin

Hong Zhou and Markus J. Seibel

There is mounting evidence that osteoblasts are involved in the regulation of global energy metabolism. Although osteocalcin signalling remains important, the complexity of systemic energy storage and expenditure makes it likely that hitherto unknown factors are also involved in osteoblast-mediated regulation of metabolism.

Refers to Yao, Q. *et al.* Wnt/β-catenin signalling in osteoblasts regulates global energy metabolism. *Bone* **97**, 175–183 (2017)

Apart from its mechanical function, the skeleton acts as an endocrine organ with regulatory roles in diverse body functions. The past few years have seen the emergence of convincing evidence indicating that bone cells are not only responsible for skeletal remodelling and homeostasis, but are also involved in the control of systemic energy metabolism. Data from several groups worldwide have shown that osteoblasts (cells that form new bone) are intimately involved in the regulation of glucose tolerance, insulin production and sensitivity, fat metabolism and global energy expenditure[1–4]. Now, new research by Yao *et al.*[5] is helping to shed light on the intricacies of osteoblast involvement in global energy metabolism.

Many of the functions of osteoblasts are thought to be mediated through the action of osteocalcin[1,2], a protein secreted exclusively by mature osteoblasts and osteocytes. Osteocalcin-knockout mice are characterized by visceral obesity, hyperglycaemia, hypoinsulinaemia, peripheral insulin resistance, glucose intolerance and low energy expenditure[1]. Mice that lack the insulin receptor on mature osteoblasts and osteocytes not only exhibit reduced bone formation (due to inhibition of osteoblast differentiation), but also develop insulin resistance, glucose intolerance and progressive fat accumulation, along with reduced energy expenditure[2]. The metabolic phenotype of these mice is similar to that seen in osteocalcin-deficient mice. In another model, mice in which the osteoblast is protected from the action of glucocorticoids, and hence the suppression of osteocalcin synthesis, do not develop the typical adverse metabolic effects of glucocorticoid treatment such as insulin resistance, diabetes mellitus and obesity[3]. Indeed, the effects of therapeutic glucocorticoids on systemic energy metabolism can be largely reversed in normal mice by replacing circulating osteocalcin via gene therapy[3].

Some, but certainly not all, clinical studies in humans report evidence that points towards a regulatory role for osteocalcin in energy metabolism. For example, high serum osteocalcin concentrations are associated with low fasting blood glucose levels[6], increased insulin sensitivity[7] and high insulin and adipokine levels[7], as well as low body fat mass[8]. However, other studies have found no association between serum osteocalcin levels and metabolic parameters in either healthy individuals or patients with diabetes mellitus[8].

Considering the complexity of systemic energy metabolism and the intricate balance between energy storage and energy expenditure, it is conceivable that other, hitherto unknown factors are involved in mediating skeletal control of these processes. Indeed, several studies, including the work of Yao *et al.*[5], suggest exactly that. For example, although showing signs of hypoinsulinaemia, glucose intolerance and reduced insulin sensitivity, mice in which the skeleton has been partially depleted of osteoblasts are characterized by substantially reduced fat mass and increased energy expenditure[4]. These effects contrast with those seen in osteocalcin-deficient mice and are not reversed by osteocalcin treatment[4]. Furthermore, mice lacking *Lrp5* (which codes for a molecule involved in the Wnt–β-catenin signalling pathway) in osteoblasts and osteocytes exhibit normal serum osteocalcin levels, reduced bone mass, increased body fat and a corresponding reduction in energy expenditure[9]. These results point to a mechanism for skeletal regulation of systemic energy metabolism that is independent of osteocalcin.

Given the incongruent results from experimental (and clinical) studies, it is worth looking closer at exactly what happens in each model. Osteocalcin, *Lrp5* and insulin receptor knockout mice each have increased fat accrual and reduced energy expenditure, whereas the removal of osteoblasts or the deletion of β-catenin in osteoblasts results in exactly opposite effects. As deletion of β-catenin or *Lrp5* in osteoblasts ultimately leads to a disruption of Wnt–β-catenin signalling in these cells, one would have expected both knockout models to have similar phenotypes. Surprisingly, this is not the case. Closer examination of the various experimental models published so far reveals that these glaring discrepancies might be caused by differences in target cell populations. Specifically, it seems that targeting cells of the early osteoblast lineage (by β-catenin knockout or osteoblast depletion) results in a phenotype of reduced fat mass and high energy expenditure. By contrast, targeting mature osteoblasts and osteocytes (by knock-out of osteocalcin, insulin receptor or *Lrp5*) leads to increased fat mass and low energy expenditure.

Yao *et al.*[5] reported not only impaired osteoblast differentiation (and hence low bone mass) in mice lacking β-catenin specifically in osteoblasts, but also a reduced number of pancreatic islets, small β cells, reduced serum osteocalcin and insulin levels, hyperglycaemia and glucose intolerance. However, unlike osteocalcin-knockout mice[1], but similar to osteoblast-depleted mice[4], these animals exhibited decreased body fat mass and increased energy expenditure[5]. Long-term treatment of these mice with osteocalcin corrected the hyperglycaemic phenotype, thus confirming a role for osteocalcin in pancreatic insulin secretion and peripheral glucose handling. Unfortunately, the authors did not report whether administration of osteocalcin to β-catenin-knockout mice had any effect on adipose tissue accrual or energy expenditure. Interestingly, however, overexpression of osteoprotegerin in the osteoblasts of β-catenin-knockout mice normalized not only their bone mass, but also their body fat mass and energy expenditure to levels seen in wild-type mice[5]. Again, the authors did not report the effects of osteoprotegerin overexpression on glucose balance and insulin secretion in this model.

Despite these limitations, Yao *et al.*[5] not only confirmed a role for osteocalcin in glucose metabolism, but also suggested a previously unrecognized effect of bone mass in the regulation of fat accrual and energy expenditure. Whether this effect is attributable to the inhibition of osteoclastogenesis and bone resorption through osteoprotegerin (and thereby related to osteoclast rather than osteoblast activity) or is mediated via osteoprotegerin and/or osteoblasts remains to be seen. However, these exciting results open up new avenues to explore the relationship between bone cells and energy metabolism. Certainly, the hunt is on to identify the key players in this relationship.

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Competing interests statement
The authors declare no competing interests.

RHEUMATOID ARTHRITIS

The ins and outs of platelets in RA

Isabel Andia

New insights into the ability of platelets to modify lymphocyte biology suggest a potential anti-inflammatory role for platelet therapy in rheumatoid arthritis. The success of this therapy will depend on researchers being able to define the best formulation to manipulate the crosstalk between inflammatory, vascular and synovial cells.

Refers to Zamora, C. *et al.* Binding of platelets to lymphocytes: a potential anti-inflammatory therapy in rheumatoid arthritis. *J. Immunol.* http://dx.doi.org/10.4049/jimmunol.1601708 (2017)

Platelets have attracted substantial attention in the past decade owing to their unforeseen immunomodulatory properties and obviously conflicting functions. In reality, platelets participate in both health and disease, having important roles not only in haemostasis and thrombotic diseases, but also in wound healing, cancer, inflammation and immunity. Contrary to previous knowledge, new insights into platelet–lymphocyte interactions suggest an unexpected role for platelets in dampening inflammation in rheumatoid arthritis (RA). In a new study, Zamora and colleagues[1] report that platelets reduce inflammation by binding to $CD4^+$ and $CD8^+$ T lymphocytes through the interaction of P-selectin (CD62P) and P-selectin glycoprotein ligand 1 (PSGL1), consequently decreasing T cell proliferation.

Zamora *et al.*[1] noted changes in the expression of cytokines (TNF, IFNγ and IL-17) by T cells upon co-culture with platelets, which were attributed to limited T cell differentiation towards a type 17 T helper (T_H17) cell phenotype. Interaction via P-selectin was of paramount importance for the immunomodulation of T cells by platelets; however, other immunosuppressive actions were mediated by the release of soluble bioactive proteins such as platelet factor 4 (PF4; also known as CXCL4), transforming growth factor β and soluble CD40 ligand (sCD40L)[1]. In addition, when mononuclear cells from the synovial fluid of patients with RA were co-cultured with homologous platelets, there was a reduction in the production of inflammatory or angiogenic cytokines (including TNF, IFNγ and IL-17), along with an increase in the anti-inflammatory cytokine IL-10 (REF. 1).

Previously, Zamora and colleagues[2] identified a specific platelet marker (CD41a) on the surface of lymphocytes from the peripheral blood of healthy individuals, and confirmed by confocal microscopy that CD41a was actually expressed by platelets that were attached to $CD36^+$ lymphocytes. Moreover, in patients with RA, the frequency of platelet-bound leukocytes was associated with disease activity scores[2]. Zamora *et al.* hypothesized that platelet–leukocyte interactions could participate in regulation of disease. Lymphocytes, monocytes, macrophages and neutrophils all interact with platelets via P-selectin, platelet glycoprotein Ibα (GPIbα) and CD40L, but the importance of these interactions in RA remains to be ascertained.

The results from the latest study by Zamora *et al.*[1] are in accordance with previous research in which intra-articular injections of platelet-rich plasma (PRP) into the joints of pigs with antigen-induced arthritis attenuated arthritic changes in both the cartilage and synovium[3]. Lippross *et al.*[3] attributed the therapeutic effects of PRP to the release of ≥300 bioactive proteins (including modulatory cytokines, interleukins and growth factors) from the platelets into the intra-articular space and the subsequent downstream activation of synovial cells and cartilage cells. Accordingly, Zamora *et al.*[1] reported that when activated platelets were separated from lymphocytes, the proteins released from the platelets (such as PF4 and sCD40L) were partially responsible for the inhibition of T cell function.

Certainly, the complex nature of platelets requires that merely studying the interaction between the platelet secretome and stromal mesenchymal cells or inflammatory cells is insufficient to fully understand the role of platelets in inflammation and immunity. Given that all inflammatory reactions in the joints of patients with RA take place within an environment containing fibroblast-like synoviocytes (FLSs), co-culturing synovial fluid cells with FLSs from the joints of patients with RA in the presence of activated platelets could help to ascertain whether platelet therapies are a promising approach for treating RA (FIG. 1).

PRP was initially used in the context of physiological healing and tissue regeneration but, as PRP is considered to be safe, its clinical use has extended to a variety of chronic conditions, including not only degenerative diseases such as osteoarthritis (OA)[4,5] but also chronic autoimmune diseases such as psoriasis[6]. Although the extent of inflammatory involvement in OA is low compared with RA, a meta-analysis from 2017 of ten randomized controlled trials in OA revealed that intra-articular injections of PRP are safe and are more beneficial for pain relief and functional recovery than hyaluronic acid and saline[5]. On the basis of the findings of Zamora *et al.*[1], the effects of platelets (probably in combination with other DMARDs) could also be explored for the management of RA.

However, platelets are multifaceted: not only do they participate in multiple processes (including cellular migration, proliferation and anabolism), but within these processes platelets can have opposing effects. High levels of platelet reactivity and increased amounts of platelet microparticles containing an inflammatory cargo (IL-1α and IL-1β) can be found in the serum and synovial fluid of patients with RA, but not in the joints of patients with OA[7]. Based partly on these observations and partly on the fact that platelet depletion in a mouse model hindered the development of inflammatory arthritis, Boilard *et al.*[7] proposed an active role for platelets in the

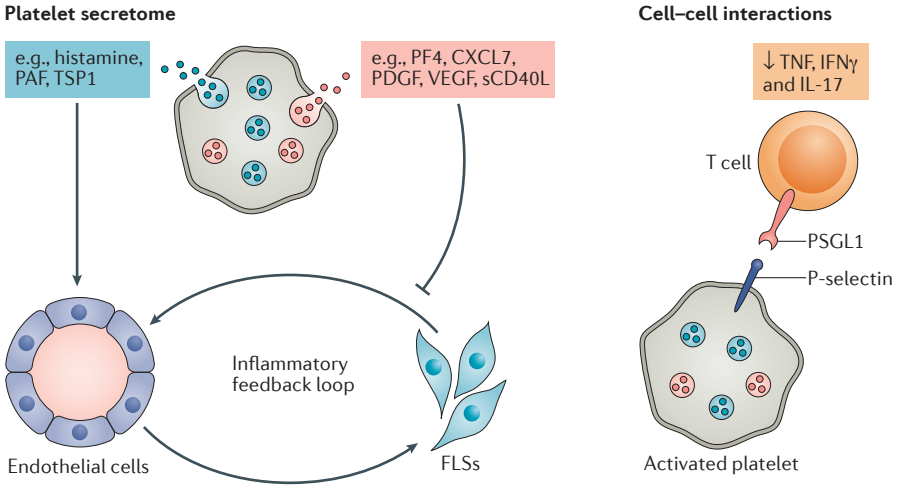

Figure 1 | **Potential anti-inflammatory mechanisms for platelet therapy in rheumatoid arthritis.** Upon activation, platelets deliver a collection of bioactive molecules that have important roles in inflammation, angiogenesis, cell migration and metabolism and can modify the inflammatory feedback loop between fibroblast-like synoviocytes (FLSs) and the synovial vasculature. In addition, platelet–lymphocyte interactions via P-selectin and P-selectin glycoprotein ligand 1 (PSGL1) might participate in disease regulation by decreasing the production of inflammatory cytokines, including TNF, IFNγ and IL-17 and increasing the production of anti-inflammatory IL-10. PAF, platelet-activating factor; PDGF, platelet-derived growth factor; PF4, platelet factor 4; sCD40L, soluble CD40 ligand; TSP1, thrombospondin 1; VEGF, vascular endothelial growth factor.

pathogenesis of RA via the promotion of leukocyte recruitment in the joint vasculature. Among the platelet-released factors that can disrupt inter-endothelial junctions are factors that are not specific to platelets, such as vascular endothelial growth factor (VEGF) and platelet-activating factor (PAF), which are stored in alpha granules, and serotonin, which is captured in the blood stream via the serotonin transporter and is either stored in dense granules or degraded by monoamine oxidase. In particular, platelet serotonin in patients with RA mediates the platelet glycoprotein VI-dependent pro-permeability effect of platelets in the inflamed joint[8]. Other molecules released from dense granules, including histamine and catecholamines (such as noradrenaline and dopamine) have the potential to modify vascular tone but require further investigation.

As synovial inflammation is associated with cartilage degradation, suppressing leukocyte extravasation and angiogenesis in the chronically inflamed synovium is a primary target for therapy. However, platelets seem to have contradictory roles in leukocyte transmigration. For example, the platelet-released chemokine β-thromboglobulin (an isoform of CXCL7) has a major role in neutrophil recruitment[4], whereas the platelet-released proresolvin protein annexin A1 hampers neutrophil traffic during inflammation[9]. Platelet degranulation also increases the generation of lipoxin A4 and maresin 1 (proresolvin mediators that limit neutrophil influx and enhance macrophage engulfment of neutrophils) following leukocyte–platelet interactions[9]. These obviously conflicting functions of platelets might be essential for maintenance of tissue homeostasis.

The role of platelets in angiogenesis is also contradictory. Platelets can mediate the secretion of proangiogenic mediators such as platelet-derived growth factor, basic fibroblast growth factor, VEGF, CXCL12 and angiopoietin. Remarkably, platelets constitute the largest reservoir of thrombospondin 1 (which inhibits endothelial cell proliferation and stimulates apoptosis) in humans (3.5 ± 0.5 μg per 10^8 platelets)[10]. In addition, PF4 (the most abundant cytokine in alpha granules, present at micromolar concentrations) is potently anti-angiogenic as it prevents VEGF from binding to endothelial cells. Identifying key molecules involved in platelet duality could limit angiogenesis and help to regulate the resolution of inflammation in RA.

So, how much closer does the work of Zamora *et al.*[1], along with previous knowledge, bring us to an effective potential intra-articular therapy for patients with RA? The hypothesis of adding platelets to the RA joint raises many complex questions, especially as platelets are activated during RA. Considering that systemic inflammation could affect megakaryocytes and thereby alter the molecular panel passed on to the budding platelets, it seems plausible that such a therapeutic approach would need to be allogeneic to avoid exacerbation of RA. Overall, it is the safety profile of intra-articular administration of platelets that will influence at which point in the course of RA clinicians might consider this new approach, yet these results are encouraging enough to merit further investigation.

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SPONDYLOARTHROPATHIES

How should axial spondyloarthritis be diagnosed?

Jürgen Braun and Uta Kiltz

Results from a cohort study are challenging the diagnostic algorithm proposed by the Assessment of Spondyloarthritis International Society by showing that rheumatologists are not always confirming a diagnosis of axial spondyloarthritis in patients with multiple features of spondyloarthritis. How will these results affect the future development of classification criteria?

Refers to Ez-Zaitouni, Z. *et al.* Presence of multiple spondyloarthritis (SpA) features is important but not sufficient for a diagnosis of axial spondyloarthritis: data from the SPondyloArthritis Caught Early (SPACE) cohort. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2016-210119 (2017)

Axial spondyloarthritis (axSpA) is a chronic rheumatic disease that causes inflammation and new bone formation in the axial skeleton. Cohorts such as the German Spondlyoarthritis Inception Cohort (GESPIC)[1], the Spondyloarthritis Caught Early (SPACE) cohort[2] and the Devenir des Spondylarthopathies Indifférenciées Récentes (DESIR) cohort[3] have contributed substantially to our understanding of axSpA, especially the early stages of disease in which inflammatory back pain due to sacroiliitis is the leading clinical symptom, but diagnosis of early axSpA remains a challenge. The Assessment of Spondyloarthritis International Society (ASAS) classification criteria[4] and a diagnostic algorithm proposed by ASAS[2] are both frequently used to diagnose axSpA. However, there are problems associated with the use of classification criteria for diagnosis, which have been discussed extensively[5], including the consequences for nomenclature for diagnosis[6]. Moreover, the latest publication from the SPACE cohort by Ez-Zaitouni *et al.*[7] suggests that application of the ASAS algorithm should not replace a thorough diagnostic work-up. In this publication[7], the local rheumatologist in charge of the patient did not confirm a diagnosis of axSpA for 38% of the patients with at least three features of SpA ($n = 79$) and for 15% of the patients with at least four features ($n = 119$).

In our opinion, allowing the diagnosis to be made by the local rheumatologist is problematic, mainly because they might have limited experience with axSpA and its differential diagnosis. Local rheumatologists frequently have limited knowledge to accurately judge original images themselves, which might lead to overinterpretation of radiography and MRI findings. Such practicioners also tend to use classification criteria for diagnosis ('to be on the safe side'), often do not recognize psoriatic arthritis as part of the SpA spectrum and can mix up diagnosis with disease activity (that is, decide against a diagnosis of axSpA because of the presence of no or little current disease activity). Given that a diagnosis of axSpA was strongly associated with any positive imaging in the SPACE cohort (OR 34.3; 95% CI 17.3–67.7)[7], the erroneous interpretation of imaging would have had a major effect on the results.

As chronic back pain is the main inclusion criterion in the SPACE cohort[7], the results of this study raise an interesting question that was not addressed by Ez-Zaitouni and colleagues, namely, what was the diagnosis in patients with three or four features of SpA who were not diagnosed with axSpA? If no alternative diagnosis was made, it would be useful to understand why the rheumatologists in charge did not make a diagnosis of axSpA. Indeed, the exclusion of other diseases or obvious explanations for the leading symptom is, for example, often used in classification criteria for other diseases such as rheumatoid arthritis[8]. Imaging data from the SPACE cohort have been used to demonstrate that degenerative changes in the axial skeleton are frequent in patients with axSpA and that these degenerative changes show better

correlation with pain than do inflammatory changes[9]. Thus, one possible explanation of the data reported by Ez-Zaitouni *et al.*[7] is that some of these patients had peripheral SpA and chronic back pain as a result of mechanical causes. However, almost all patients and/or treating physicians within the cohorts with three and four features of SpA reported the presence of inflammatory back pain (90% and 94%, respectively), which does not argue in favour of a mechanical cause of back pain. On the other hand, one argument in favour of this possibile cause of pain is that in the group of patients with more than four features of SpA, there were many patients (45% of the total group) who had psoriasis and chronic inflammatory bowel disease. In addition, of the patients with at least four features of SpA, 39% had peripheral arthritis, 19% dactylitis, 65% enthesitis and 58% elevated C-reactive protein level — all symptoms indicative of peripheral SpA. However, this cohort study[7] did not compare the prevalence of extra-spinal manifestations in patients with a diagnosis of axSpA versus those without. Clearly, in patients with psoriasis and inflammatory bowel disease, HLA-B27 positivity is less important for making a diagnosis of axSpA, as the genetic backgrounds of these diseases are different.

On the basis of our experience, it is unlikely that a patient with more than four features of SpA does not have SpA. Thus, we believe that the results from this latest SPACE cohort study[7] are primarily challenging the strategy used to define the 'gold standard' for axSpA diagnosis when developing or testing classification criteria; that is, the opinion of the local rheumatologist. We should therefore develop more sophisticated ways to ascertain a diagnosis; for example, the use of an external expert panel that discusses the diagnosis with the local rheumatologist, which would then serve as the gold standard. This approach would probably reduce the inclusion of patients with a doubtful MRI finding or healthy individuals who are HLA-B27 positive. Clearly, inception cohorts are likely to include such individuals, given that in central Europe, which has an HLA-B27 prevalence of about 8%, >90% of all HLA-B27-positive individuals will not develop axSpA[10]. This proposal of a new definition of a gold standard seems to be consistent with the views of Ez-Zaitouni and colleagues[7].

So what is the take-home message of the Ez-Zaitouni *et al.* study[7] for rheumatologists with regard to routine patient care? The presented data demonstrate clearly that the likelihood of a diagnosis of axSpA increases with the number of typical SpA features. Furthermore, Ez-Zaitouni *et al.* confirm that imaging findings and HLA-B27 status are the strongest contributors to diagnosis and classification of axSpA. This latter point puts a lot of responsibility onto the shoulders of local rheumatologists, who need to be well trained in the interpretation of conventional radiographs and MRI. Radiologists with limited experience in musculoskeletal diseases might also struggle to interpret the sophisticated imaging features of axSpA. Finally, these results remind rheumatologists to ask an important question: is there another, potentially better, explanation for the symptoms reported by the patient? As the results from the Ez-Zaitouni *et al.* study[7] provide no alternative diagnoses for the patients' symptoms, we cannot be sure about the credibility of the diagnosis of 'no axSpA' in 18 patients with more than four features of SpA. In conclusion, although the ASAS diagnostic algorithm still has a place in our diagnostic strategy when assessing patients with chronic back pain and suspected axSpA, we might be able to improve the performance of classification studies by choosing a different gold standard.

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REVIEWS

Metabolic regulation of inflammation

Timo Gaber[1,2], Cindy Strehl[1,2] and Frank Buttgereit[1,2]

Abstract | Immune cells constantly patrol the body via the bloodstream and migrate into multiple tissues where they face variable and sometimes demanding environmental conditions. Nutrient and oxygen availability can vary during homeostasis, and especially during the course of an immune response, creating a demand for immune cells that are highly metabolically dynamic. As an evolutionary response, immune cells have developed different metabolic programmes to supply them with cellular energy and biomolecules, enabling them to cope with changing and challenging metabolic conditions. In the past 5 years, it has become clear that cellular metabolism affects immune cell function and differentiation, and that disease-specific metabolic configurations might provide an explanation for the dysfunctional immune responses seen in rheumatic diseases. This Review outlines the metabolic challenges faced by immune cells in states of homeostasis and inflammation, as well as the variety of metabolic configurations utilized by immune cells during differentiation and activation. Changes in cellular metabolism that contribute towards the dysfunctional immune responses seen in rheumatic diseases are also briefly discussed.

Metabolism is the fundamental process by which energy homeostasis is maintained and cells are supplied with the building blocks required for the synthesis of macromolecules. During the past few years, it has become evident that the metabolic state of an immune cell can directly influence its ability to function and differentiate, ultimately affecting immunity, tolerance and, in the case of autoimmunity, the failure of an immune response[1–4].

Different immune cell subsets have different metabolic requirements and face a variety of metabolic challenges; however, they all share the need to maintain energy homeostasis to survive and function. Once homeostasis is disturbed, for example during infection or tissue damage, signals released from infected, dying or stressed cells and tissues induce the precise and timely process of inflammation[5]. Although the end point of the inflammatory response is the resolution of inflammation and the re-establishment of homeostasis, resolution can sometimes be insufficient (as in the case of immunodeficiency) or the inflammatory response exacerbated, leading to chronic inflammation or autoimmunity. In the past 5 years, it has become evident that as well as intrinsic risk factors such as a genetic predisposition to a disease, extrinsic environmental factors such as nutritional factors and a patient's microbiota or, more precisely, their microbial metabolites, contribute to the development of autoimmunity[6–8]. Several research groups have found alterations in the metabolic configurations of cells during different autoimmune diseases with regard to metabolic enzyme activities, metabolites and intermediates and key metabolic checkpoint molecules, which could contribute to aberrant immune cell behaviour[4,9,10].

In this Review, we summarize and discuss what is known to date about immunometabolism in homeostasis and its reconfiguration during inflammatory responses, as well as discussing the effects of immunometabolism upon the resolution of inflammation in different immune cell subsets. We focus particularly on the effect of metabolic enzymes, intermediates and metabolites on inflammatory responses, and on how metabolism affects immune responses in rheumatic diseases. This Review provides an overview of immunometabolism; the specific role of immunometabolism in systemic lupus erythematosus (SLE)[11], rheumatoid arthritis (RA)[12] and osetoarthritis[13], as well as current knowledge on targeting metabolic pathways therapeutically[14] are covered in other articles in this journal.

Cellular metabolism in homeostasis

All cells need access to sufficient and appropriate nutrients and oxygen to maintain homeostasis. Most resting immune cells (such as naive T cells, resting B cells and circulating monocytes[1,2,15]), but also so-called long-lived cells (such as resting immature bone-marrow-derived dendritic cells (DCs), memory T cells and plasma B cells) are relatively metabolically inactive, with minimal biosynthetic demands beyond normal 'housekeeping' processes[16–20] (FIG. 1). Resting immune cells use energy

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Key points

- Immune cells face a variety of variable and sometimes demanding environmental conditions, requiring them to display a dynamic range of metabolic adaptation processes
- Under inflammatory conditions, stimulated immune cells have an acute need to generate sufficient energy and biomolecules to support growth, proliferation and the production of proinflammatory molecules
- Metabolic reconfiguration varies between innate and adaptive immune responses, and influences both the effector phase of inflammation and the resolution of inflammation by modulating immune cell fate and function
- Metabolic enzymes, metabolites and regulators of metabolism have a direct influence on certain inflammatory responses
- Alterations of metabolic configurations of immune cells can contribute to dysfunctional immune responses, a typical feature of autoimmunity

in the form of ATP, which is produced predominantly via aerobic metabolism. At low rates of glucose uptake, ATP is generated directly by glycolysis and indirectly by the oxidation of glucose-derived pyruvate to CO_2 via the mitochondrial tricarboxylic acid (TCA) cycle, which generates the reducing energy intermediates NADH and $FADH_2$. These reducing agents provide electrons for complexes I and II of the electron transport chain, thereby driving the process of oxidative phosphorylation. This process establishes a proton gradient across the inner mitochondrial membrane, fuelling ATP synthases and ultimately generating a maximum of 36 ATP molecules per one molecule of glucose[1]. Interestingly, long-lived memory T cells, regulatory T (T_{reg}) cells and immature bone-marrow-derived DCs can also utilize fatty acid oxidation, yielding large amounts of acetyl-CoA, NADH and $FADH_2$ to fuel the TCA cycle and oxidative phosphorylation[16–19] (FIG. 1). Inhibiting glycolysis can promote the formation of long-lived memory T cells[21]. Moreover, promoting oxidative phosphorylation in 'short-lived' immune cells, such as activated DCs, results in an increased cellular lifespan, whereas inhibiting fatty acid oxidation and oxidative phosphorylation reduces memory T cell formation[22]. These results highlight the flexibility of immune cells to adapt to different metabolic requirements.

Metabolic challenges for immune cells

Under normal conditions, most tissues are well-supplied with nutrients and oxygen by networks of blood and lymphatic vessels, so it is not surprising that most tissue-resident cells are metabolically well-adapted to these conditions. Local and systemic metabolic responses of tissue-resident cells vary with the supply of nutrients and are influenced by gut microbiota and organs such as the gut, liver and kidneys, which distribute metabolites around the body[23]. Immune cells, however, traverse a broad range of tissues, travelling from their origin (for example, bone marrow) via the bloodstream to their target tissue or to a site of immune action, eventually arriving in the lymphatic drainage system. During this process, immune cells face a variety of variable and sometimes demanding environmental conditions, requiring them to display a dynamic range of metabolic adaptation processes.

By contrast, during activity, transformation, growth, development or inflammation, local microenvironmental conditions can become metabolically challenging. During inflammation, 'front-line' resident immune cells such as macrophages and DCs become activated by signals from infected or damaged tissues. As a result, blood flow to the surrounding area increases, as does the concentration of cells within the blood, promoting the active recruitment of neutrophils and other leukocytes to the site of inflammation[23]. After activation, immune cells undergo substantial changes to support robust cell growth (for example, the transition from monocytes to macrophages), rapid cell proliferation (such as clonal T cell expansion) or new functions such as phagocytosis or enhanced release of inflammatory mediators. Competition for nutrients, oxygen and metabolites not only with other immune cells, but also with bacteria or parasites present in the host, can lead to substantial changes in conditions within the tissue microenvironment. These changes can switch accommodative metabolic conditions to hostile metabolic conditions. As a result, each immune cell subset has its own adaptive metabolic programmes, often set in motion by activation or differentiation, which enable them to cope with these hostile conditions and to fulfil specific functions during the inflammatory process.

Metabolic regulation of inflammation

Almost a century ago, Otto Warburg observed that immune cell activation, or conversion from a resting state into an effector mode, required an abrupt metabolic switch from oxidative phosphorylation to glycolysis (something that usually occurs in hypoxia), regardless of the availability of oxygen (termed aerobic glycolysis), providing energy in the form of ATP and biosynthetic precursors for cell proliferation and effector functions. This phenomenon was termed the Warburg effect[24] (FIG. 2).

Under inflammatory conditions, stimulated immune cells have an acute need to generate sufficient energy and biomolecules to support growth, proliferation and the production of proinflammatory molecules. Thus, immune cell metabolism shifts towards aerobic glycolysis. This shift funnels glucose-6-phosphate into the pentose phosphate pathway (PPP), provides 3-phosphoglycerate for the serine biosynthetic pathway (required for the synthesis of amino acids to create cytokines) and supplies pyruvate for the TCA cycle, which synthesizes citrate (an intermediate for fatty acids used in membrane assembly)[1] (FIG. 2). Moreover, metabolic reconfiguration of immune cells towards aerobic glycolysis enables these cells to better cope with metabolically restrictive inflammatory conditions, such as during the transition from normoxic to hypoxic conditions[25,26]. Notably, efficient ATP production via oxidative phosphorylation is prevented under hypoxic conditions, such as those found in areas with active inflammatory processes[27,28].

Aerobic glycolysis occurs in macrophages stimulated with IFNγ or activated via Toll-like receptors (TLRs)[29,30], TLR-activated DCs[16],phorbol 12-myristate 13-acetate-stimulated neutrophils[31], engaged natural killer cells[32,33], B cells[34], activated effector T cells[1,35] (such as

Glycolysis
An oxygen-independent metabolic pathway that generates two molecules of pyruvate, ATP and NADH from every one molecule of glucose, supporting the tricarboxylic acid cycle and providing intermediates for the pentose phosphate pathway, glycosylation reactions and the synthesis of biomolecules (including serine, glycine, alanine and acetyl-CoA)

Tricarboxylic acid (TCA) cycle
(Also known as the Krebs cycle) A set of connected pathways in the mitochondrial matrix, which metabolize acetyl-CoA derived from glycolysis or fatty acid oxidation, producing NADH and $FADH_2$ for the electron transport chain and precursors for amino acid and fatty acid synthesis

Electron transport chain
A series of proteins in the inner mitochondrial membrane that transfer electrons from one to the other in a series of redox reactions, resulting in the synthesis of ATP and in the movement of protons out of the mitochondrial matrix

Oxidative phosphorylation
A metabolic pathway that produces ATP from the oxidation of acetyl-CoA and the transfer of electrons to the electron transport chain via NADH and $FADH_2$

Fatty acid oxidation
A metabolic process that produces ATP from the oxidation of acetyl-CoA derived from the mobilization of fatty acids

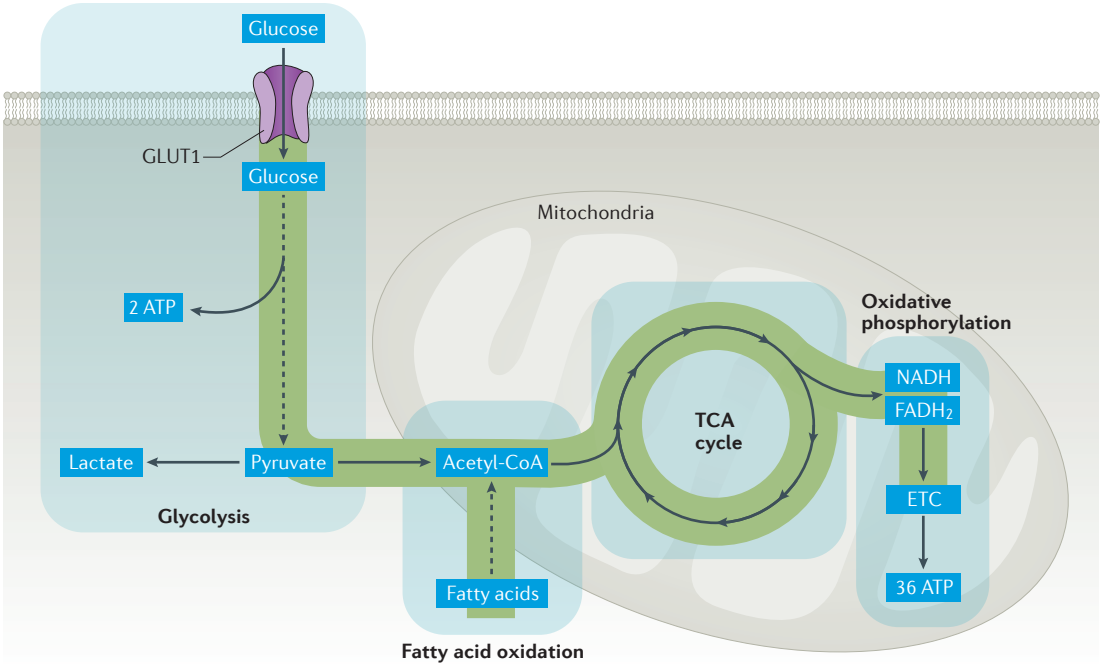


Figure 1 | **Immune cell metabolism during homeostasis.** Immune cells need energy in the form of ATP to survive, grow, reproduce and perform specific functions. Glucose is imported into the cytosol via glucose transporter type 1 (GLUT1), where it is converted to pyruvate over a series of enzymatic steps known as glycolysis. After transfer to the mitochondria, pyruvate is converted to the tricarboxylic acid (TCA) cycle substrate, acetyl-CoA. In resting, long-lived immune cells, such as regulatory T cells or memory T cells, acetyl-CoA is also produced by fatty acid oxidation. Pyruvate is ultimately metabolized to CO_2, generating NADH and $FADH_2$. These two reducing agents drive the electron transport chain (ETC) in a process called oxidative phosphorylation, by donating electrons and providing hydrogen molecules for the creation of water. This process builds a proton gradient that ultimately generates ATP through the phosphorylation of ADP.

$CD4^+$ T helper (T_H) cells[17,36]) and $CD8^+$ effector T cells[37]. Shifting immune cell metabolism towards aerobic glycolysis begins with an increased expression of the facilitators of glucose transport, such as glucose transporter type 1 (GLUT1), enabling the efficient uptake of glucose in an environment in which the supply of nutrients is restricted by inflammation[38]. Although glucose is an essential fuel for immune cell activation, effector cells can instantly adapt to low glucose levels by increasing their uptake of glutamine and initiating glutaminolysis to keep the TCA cycle going[30] (FIG. 2). Moreover, effector T-cell differentiation and function are impaired when glutamine supply is abolished, shifting effector T cells from a type 1 T helper (T_H1) cell fate towards a T_{reg} cell phenotype[39–41]. Alongside glutamine, T cells can also generate metabolic intermediates through the uptake of several other amino acids such as arginine and leucine, which are required for optimal effector T-cell function[42,43].

Immunometabolism during inflammation

Lymphoid cells

T cells. During inflammation, professional antigen-presenting cells (such as macrophages and DCs) engage the adaptive immune system by activating metabolically quiescent naive T cells. Upon activation, naive T cells switch from oxidative phosphorylation to aerobic glycolysis, increasing their glucose uptake (for example by increasing the amount of GLUT1 at the cell surface)[38]. Glucose is the main source of carbon for the biosynthetic pathways producing nucleotides, amino acids and lipids that aid the massive clonal expansion of antigen-specific T cells[38,44], with T cells that lack GLUT1 failing to increase glycolysis, grow or proliferate following activation[38]. This initial step of metabolic reconfiguration is mainly regulated by mechanistic target of rapamycin (mTOR), the catalytic subunit of two distinct complexes, mTOR complex 1 (mTORC1) and mTORC2. mTOR maintains levels of the transcription factor Myc proto-oncogene protein, leading to Myc-mediated expression of glycolytic genes[42,45,46]. Subsequently, T cells differentiate into functionally distinct subsets, possessing unique metabolic configurations that are essential for their function; mTORC1 is required for differentiation of T_H1 cells and type 17 T helper (T_H17) cells, whereas mTORC2 is required for type 2 T helper (T_H2) cell differentiation[47]. Furthermore, in T_{reg} cells and memory T cells, mTOR activity has to be low for the cells to switch back to an oxidative metabolic state[48]. Effector T cell subsets ($CD4^+$ T_H1, T_H2 and T_H17 cells and $CD8^+$ cytotoxic T cells) induce the Warburg effect upon activation and rely mainly on aerobic glycolysis and the PPP with some glutaminolysis to support cellular metabolism, although still utilizing oxidative phosphorylation to fuel proliferation[17,44,45,49].

By contrast, T_{reg} cells are more likely than effector T cells to depend on oxidation of low levels of glucose and to fuel oxidative phosphorylation via fatty acid oxidation using exogenously derived fatty acids[17,36,50]. Indeed, blocking glycolysis inhibits proinflammatory

Glutaminolysis
The metabolic process by which glutamine is metabolized to glutamate and then to α-ketoglutarate to replenish the tricarboxylic acid cycle

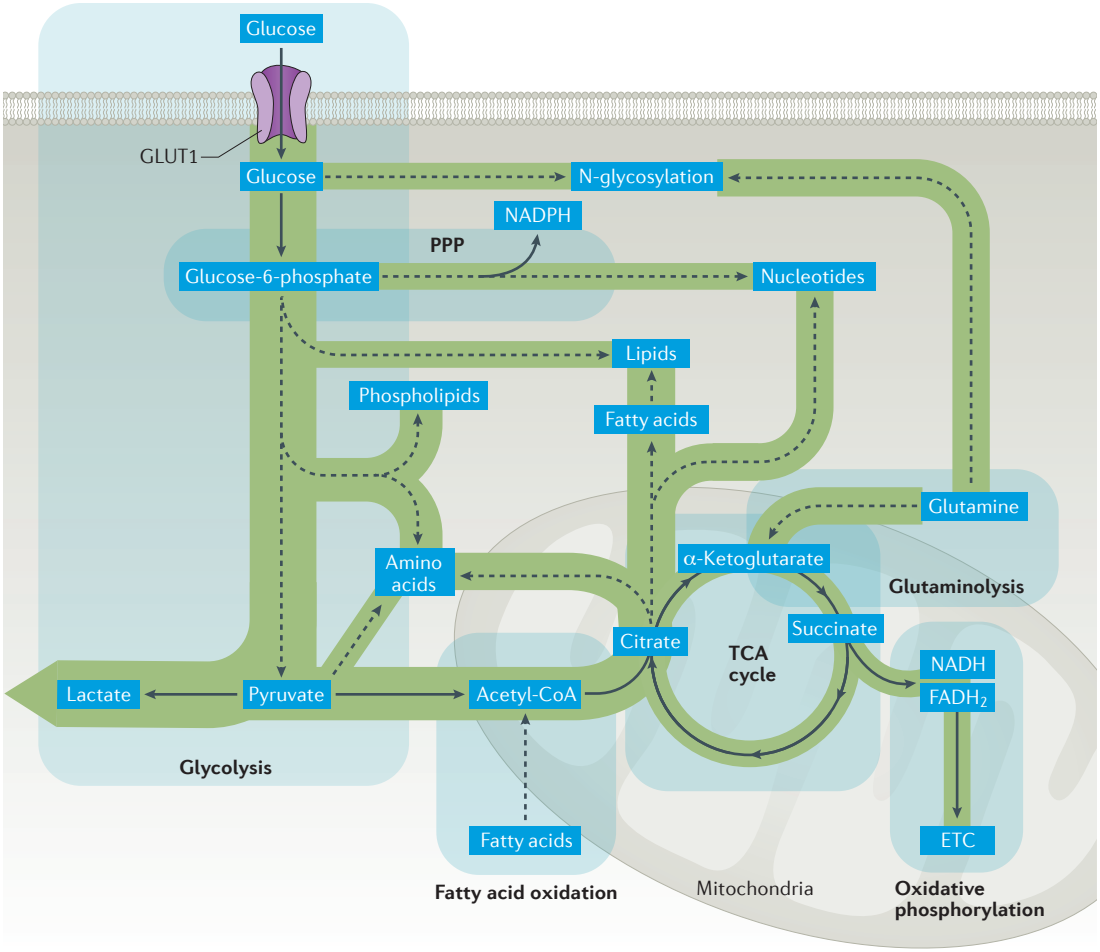


Figure 2 | **Metabolic reprogramming of immune cells upon activation.** During inflammation, immune cells are activated and convert from a resting state into an effector mode, reprogramming their metabolism to aerobic glycolysis. An increase in glucose transportation into the cell drives elevated glycolytic activity, causing excessive availability of glycolytic intermediates, which serve as precursor molecules for biosynthetic processes. For example, glucose 6-phosphate (generated by the first step in glycolysis) can feed into the pentose phosphate pathway (PPP), supporting nucleotide synthesis and the generation of NADPH. Another example is cytoplasmic acetyl-CoA (generated from glucose via pyruvate), which supports the production of cholesterol and fatty acids for lipid synthesis. Of note, many pyruvate molecules are converted to lactate, which is secreted from cells and can substantially affect the pH of the surrounding milieu. Although aerobic glycolysis is an inefficient way to generate ATP (creating only two molecules of ATP per molecule of glucose), high rates of flux through this pathway enables energy homeostasis to be sustained, even when mitochondrial ATP synthesis is impaired. Alternative fuels such as glutamine feed into the tricarboxylic acid (TCA) cycle and supply biomolecules for biosynthetic processes. ETC, electron transport chain; GLUT1, glucose transporter type 1.

T_H17 cell development but promotes the generation of anti-inflammatory T_{reg} cells[36]. Moreover, T_H17 cells can utilize *de novo* fatty acid synthesis, a process also observed in classically activated macrophages[51–54]. Inhibition of *de novo* fatty acid synthesis in T_H17 cells promotes the development of T_{reg} cells[51]. The proliferative and suppressive capacities of T_{reg} cells are also kept in balance by T_{reg} cell metabolism. TLR ligation-mediated increases in glycolysis, anabolic metabolism and mTORC1 activity reduced the suppressive function of T_{reg} cells, but increased their proliferation[55]. Conversely, these effects were opposed by FOXP3 expression, which led to increased use of oxidative and catabolic pathways[55].

An interesting proteome-based study focusing on conventional $CD4^+$ T cells and $FOXP3^+$ T_{reg} cells shed new light on T-cell metabolism in *ex vivo* and *in vitro* cultured T cells[56]. *Ex vivo* T_{reg} cells were highly glycolytic, whereas T_{reg} cells engaged both glycolysis and fatty acid oxidation to proliferate *in vitro*[56], as observed previously in other studies[17,55,57]. By contrast, *ex vivo* conventional T cells predominantly utilized fatty acid oxidation, whereas conventional T cell proliferation mainly relied on glycolysis *in vitro*[56], again, in agreement with previous studies[17,57]. The differences in metabolism between T cell subtypes might reflect bias caused by *in vitro* manipulation, which should be addressed by further research.

The final step in generating an adaptive memory following antigen challenge involves memory T cells undergoing further metabolic re-configuration towards oxidative metabolism, thereby downregulating glycolysis and inducing fatty acid oxidation to efficiently produce energy[18]. Furthermore, memory T cells also induce

mitochondrial biogenesis[19]. Thus, the ability to recall an immune response following a second activation — the primary principle of immune memory — is granted by the ability of memory T cells to rapidly induce aerobic glycolysis[18,19,37].

B cells. In contrast to the wealth of information available regarding the regulation of cellular metabolism in T cell subsets, information about B cell subsets is scarce. Like T cells, B cells also undergo metabolic reprogramming upon activation, transitioning from naive quiescent cells to activated anabolic cells. This metabolic shift results in rapid growth, proliferation and differentiation, thereby increasing not only glucose uptake and aerobic glycolysis, but also glutaminolysis and oxidative phosphorylation in equal measure[34,58,59].

The classical B cell response begins when antigen-experienced $CD4^+$ T cells engage B cells via CD40, initiating further differentiation[60]. Once activated, B cells undergo antigen-driven clonal expansion and secondary rearrangement of their immunoglobulin repertoire, a process that takes place in the germinal centre, a poorly vascularized site of intense cell activity and proliferation within lymphoid structures[61]. Thus, germinal centre B cells face increased metabolic demands while in a restrictive microenvironment, which inevitably leads to germinal centre hypoxia[62]. Although germinal centre hypoxia accelerates class switch recombination and plasma cell formation[63], sustained hypoxia or expression of hypoxia-inducible factors limits mTORC1, which is essential for B cell development and B cell clonal expansion[64]. These findings suggest that a regional variation in hypoxia is essential for B cell survival and function[62]. However, germinal centre B cells can adapt their metabolic programme to respond to a hypoxic environment by increasing mitochondrial biogenesis, glucose uptake and hypoxia-inducible factor 1α (HIF-1α)-dependent glycolysis[15]. Limiting glycolysis by providing cells with the non-metabolizable glucose analogue 2-deoxy-D-glucose (2-DG)[15] or by deleting *GLUT1* (REF. 58) results in decreased numbers of mature germinal centre B cells and reduced T cell-dependent IgM and IgG antibody production. Durable antibody-producing B cells (so-called long-lived plasma cells) also enhance their survival capacity by increasing their rate of glucose import[20]. Only a small amount of this glucose is used to sustain glycolysis and mitochondrial pyruvate import (suggesting the use of oxidative phosphorylation), whereas most is required for antibody glycosylation[20].

Aside from classical B cell activation involving B cell receptor and CD40 engagement, a variety of stimuli such as TLR ligands and cytokines can induce the metabolic reprogramming of B cells towards aerobic glycolysis via different signalling pathways that culminate in mTORC1, Myc or signal transducer and activator of transcription 6 (STAT6) signalling[34,58,65–68]. In a 2017 study, glycogen synthase kinase 3 was identified as a 'metabolic checkpoint regulator' in B cells[15]. Glycogen synthase kinase 3 acts via several mechanisms in anti-CD40-stimulated and IL-4-stimulated B cells, including restricting cell mass accumulation, promoting maintenance of a quiescent state in naive recirculating B cells, repressing Myc-dependent cell growth, restricting metabolic activity, repressing proliferation and reducing reactive oxygen species (ROS)-induced apoptosis in response to nutrient stress and hypoxia[15].

Myeloid cells

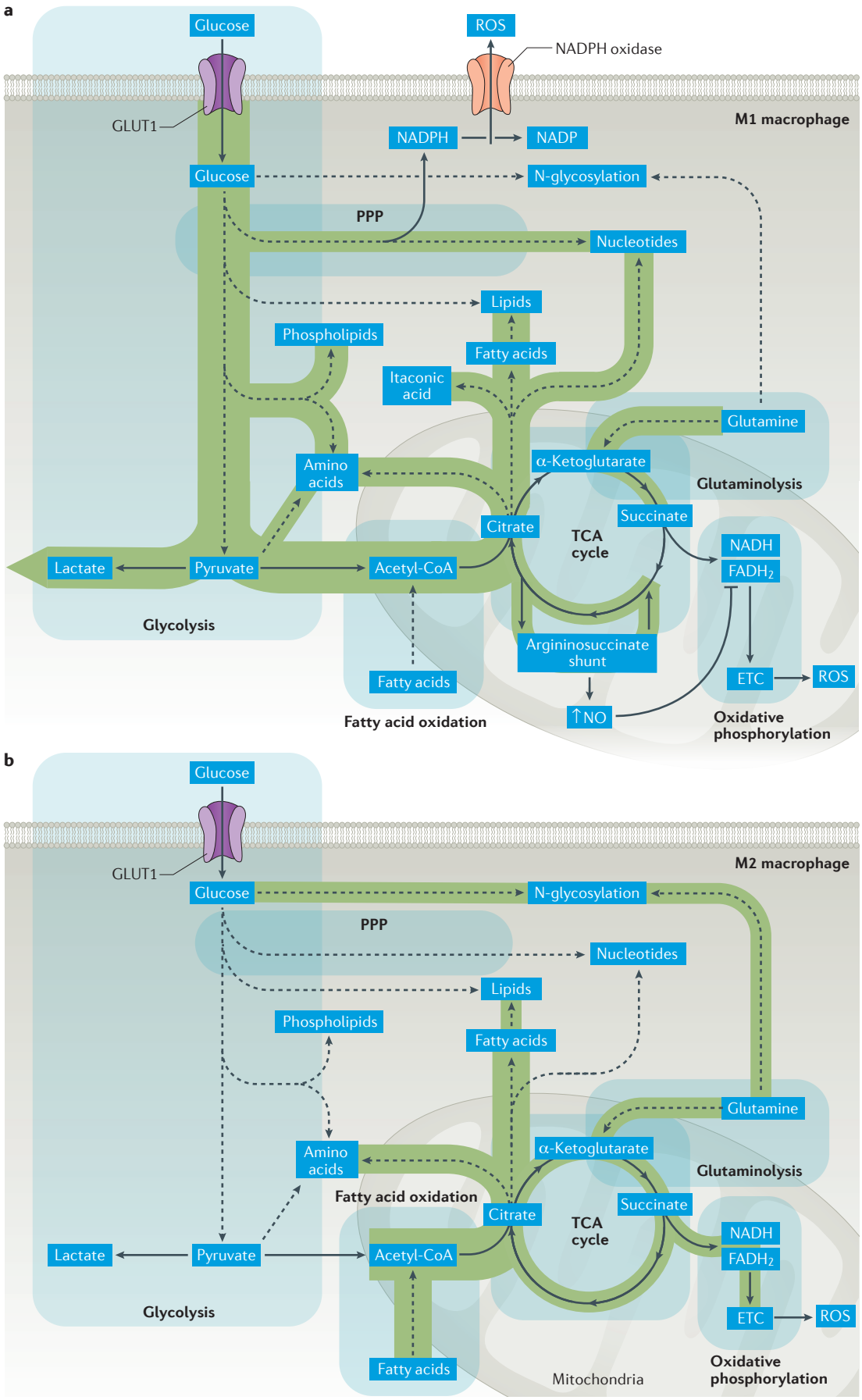
Initiation of inflammation is induced by macrophages, mast cells and DCs, which act as sentinels. Upon activation, these cells initiate well-controlled programmes to direct inflammatory processes that result in increased permeability of local blood vessels, release of chemokines and recruitment of neutrophils[69]. Macrophages, DCs and neutrophils are mature, terminally differentiated myeloid cells. In contrast to lymphocytes, these cells do not tend to proliferate, but are highly phagocytic and secrete a variety of immune mediators.

M1 macrophages. Macrophages can be polarized into distinct subsets, including proinflammatory classically activated (also known as M1) macrophages and alternatively activated (also known as M2) macrophages. Monocytes differentiate into M1 macrophages upon activation via pattern-recognition receptors or proinflammatory cytokines[70]. Once activated, M1 macrophages are highly microbicidal, release ROS (for example hydroxide) and reactive nitrogen species (such as nitric oxide (NO)), and secrete large amounts of proinflammatory cytokines such as TNF, IL-1β and IL-6.

Activated M1 macrophages (and DCs) rely primarily on glycolysis, expressing high levels of the rate-limiting glycolytic activator 6-phosphofructo 2-kinase/fructose-2, 6-bisphosphatase 3 (PFKFB3), but have reduced flux through the electron transport chain and almost no oxidative phosphorylation[52] (FIG. 3a). Hypoxic conditions caused by the inflammatory process and inducible nitric oxide synthase (iNOS)-dependent NO production directly inhibit electron flux through the electron transport chain, abolishing oxidative phosphorylation[52]. As a result, the TCA cycle halts and intermediates such as citrate and succinate accumulate[52]. Citrate is exported from the mitochondria into the cytosol, where it is converted to acetyl-CoA, an important intermediate for several biosynthetic pathways such as the synthesis of fatty acids and the production of antimicrobial and proinflammatory molecules (including itaconic acid, NO, ROS and prostaglandins)[52–54] (FIG. 3a).

Succinate is oxidized by succinate dehydrogenase, thereby elevating mitochondrial ROS levels[71]. The presence of succinate and its oxidation by-product, ROS, leads to the stabilization and increased activity of HIF-1α[52,53,71,72], the oxygen-sensitive α-subunit of the transcription factor HIF-1, which is stable under hypoxic conditions and following lipopolysaccharide (LPS) stimulation, and which dimerizes with the constitutively expressed HIF-1 β-subunit[73]. Once stabilized, HIF-1 transactivates target genes that support the Warburg effect, and, in M1 macrophages, that sustain IL-1β production[71,72].

One key feature of M1 macrophages is their ability to produce high levels of ROS and reactive nitrogen species, either via an inhibited or incomplete mitochondrial



◀ Figure 3 | **Metabolic configurations of M1 and M2 macrophages. a** | Classically activated M1 macrophages primarily use aerobic glycolysis to generate energy and molecules for biosynthetic processes, thereby increasing flux through the pentose phosphate pathway (PPP), leading to an increase in NADPH (an energy substrate for redox homeostasis and for the production of reactive oxygen species (ROS) by NADPH oxidases). ROS are also produced by mitochondria. Although the tricarboxylic acid (TCA) cycle is mainly fuelled by pyruvate from glycolysis and acetyl-CoA or succinyl-CoA from fatty acid oxidation, TCA intermediates removed from the mitochondria for use in biosynthetic pathways have to be replenished by glutamine in M1 macrophages due to the interruption of the TCA cycle at two positions. The first break in the TCA cycle leads to the accumulation of citrate, a substrate for the synthesis of itaconic acid and fatty acids (including the generation of prostaglandins). Anabolic synthesis of fatty acids generates NADPH and, subsequently, ROS via NADPH oxidases. The second break leads to the accumulation of succinate, which activates hypoxia-inducible factor 1α-induced inflammatory gene expression and increases glycolysis. The argininosuccinate shunt replenishes levels of fumarate and malate, required for citrate production, thereby generating nitric oxide (NO), which inhibits succinate dehydrogenase activity. **b** | Alternatively activated M2 macrophages are characterized by increased fatty acid oxidation, low glycolysis activity and reduced flux through the PPP. Acetyl-CoA generated by fatty acid oxidation enters the intact TCA cycle for oxidative metabolism. Glutamine is mainly used for the synthesis of amino-sugars and nucleotide sugars, but also fuels the TCA cycle. ETC, electron transport chain; GLUT1, glucose transporter type 1.

electron transport chain[74], or via NADPH oxidase and iNOS during the elimination of phagocytosed pathogens[75]. NADPH oxidase and iNOS utilize NADPH as a substrate, which is produced by the PPP or by the conversion of malate to pyruvate by malate dehydrogenase[54,75,76]. Moreover, NADPH is also used by M1 macrophages for phagocytosis and for the production of the antioxidant glutathione, which protects the cell against ROS-mediated damage[76]. Phagocytosis requires a large turnover of lipids for the generation of membranes, which is facilitated by the metabolic provision of citrate and NADPH, essential substrates for fatty acid synthesis[77]. Thus, an increase in glycolysis, reduction in oxidative phosphorylation, inhibition of fatty acid oxidation and induction of fatty acid synthesis act together to facilitate the differentiation of proinflammatory M1 macrophages (FIG. 3a).

M2 macrophages. Alternatively activated (M2) macrophages, have an anti-inflammatory phenotype and low antigen-presentation capacity, and are associated with tissue regeneration and angiogenesis. M2 macrophages have an important role in the resolution of inflammation and differ substantially in their metabolic configuration from M1 macrophages[70].

In contrast to the relatively short-lived M1 macrophages, sustained oxidative phosphorylation, glucose deprivation and the presence of free fatty acids promote a shift from proinflammatory to anti-inflammatory macrophage functions, and towards differentiation into longer-lived M2 macrophages[30,52,76,78–80] (FIG. 3b). M2 macrophages express low levels of PFKFB3 and high levels of PFKFB1, which has less kinase activity than PFKFB3, thereby reducing glycolytic flux[29]. Furthermore, M2 macrophages have high levels of arginase-1 activity, which is needed to metabolize arginine to proline, a component of collagen, thus stimulating extracellular matrix synthesis, required for tissue repair and resolution of inflammation[70,81]. Polarization of cells to an M2-like macrophage phenotype by IL-4 stimulates mitochondrial biogenesis and fatty acid oxidation, providing fuel for the intact TCA cycle as well as for oxidative phosphorylation and preparing the cells for tissue re-oxygenation following the induction of angiogenesis[82].

M2 macrophages utilize glutamine to generate TCA cycle intermediates (FIG. 3b), but also for protein glycosylation. Furthermore, M2 macrophages downregulate flux through the PPP by inducing carbohydrate kinase-like protein, which inhibits this pathway, resulting in reduced NADPH-mediated glutathione levels[76]. Notably, M2 macrophages, FOXP3$^+$ T_{reg} cells and long-lived memory T cells have metabolic features in common. FOXP3$^+$ T_{reg} cells and long-lived memory T cells also both rely on oxidative metabolism with low rates of glycolysis and fuel oxidative phosphorylation by oxidation of exogenously derived fatty acids[17–19,36,37,50].

Dendritic cells. In the initial phase of inflammation, DCs recognize invading pathogens or endogenous danger signals by the engagement of pattern-recognition receptors such as TLRs, and activate innate and adaptive immune responses by facilitating antigen-specific T cell activation[83]. Activation of DCs via TLR agonists increases glycolysis within minutes, but inhibits flux through the electron transport chain by increasing levels of NO[84]. Thus, upregulation of glycolysis in activated DCs provides these cells with molecular building blocks, *de novo* lipid synthesis for the expansion of the Golgi apparatus and the endoplasmic reticulum, as well as cellular energy required for DC effector functions[52].

Data from 2005 indicated that during maturation, DCs are also capable of storing large amounts of fat and glycogen[85]. This finding was supported by the results of a 2016 study, which showed that carbon molecules from glycogen-derived glucose feed into the TCA cycle to support early DC maturation, a critical step for early effector responses in these cells[86]. Thus, glycogen metabolism supports the activation of DCs, particularly during the period before these cells can increase their surface expression of GLUT1 (REF. 87). However, inhibition of glycolysis impairs both the survival and effector function of activated DCs, highlighting the importance of glucose as a substrate in supporting the response of DCs to TLR agonists[52].

Other myeloid cells. In contrast to macrophages and DCs, data on the metabolic configuration of mast cells is scarce. A study on mast cell metabolism from 1967 implicated a role for both glycolysis and oxidative phosphorylation in the degranulation process, since histamine release is inhibited by 2-DG[88]. In the past few years, however, research has shown that mast cell mitochondria translocate to the site of exocytosis during degranulation, suggesting an involvement of mitochondrial oxidative phosphorylation in degranulation[89,90], and implicating mitochondrial STAT3 in this process[91]. In a 2017 study, mast cell effector function, namely degranulation upon IgE and antigen stimulation through the high-affinity IgE receptor, resulted in a rapid increase in aerobic glycolysis, but not in an increase in mitochondrial respiration, although inhibition of oxidative phosphorylation drastically decreases mast cell degranulation and cytokine production[92].

The metabolic configuration of granulocytes has been best described for neutrophils and follows the same metabolic principles as M1 macrophages and tissue-resident mature DCs[69]. Primarily, neutrophils rely on glycolysis and have very low levels of oxidative phosphorylation[31,93–95]. Neutrophil effector functions such as the formation of neutrophil extracellular traps depend on glycolysis and the PPP[31,96,97].

Metabolic enzymes in inflammation

Knowing that cellular metabolism influences both the effector phase of inflammation and resolution of inflammation by modulating immune cell fate and function raises the question of whether metabolic enzymes and regulators of metabolism might have a direct influence on certain inflammatory responses (FIG. 4).

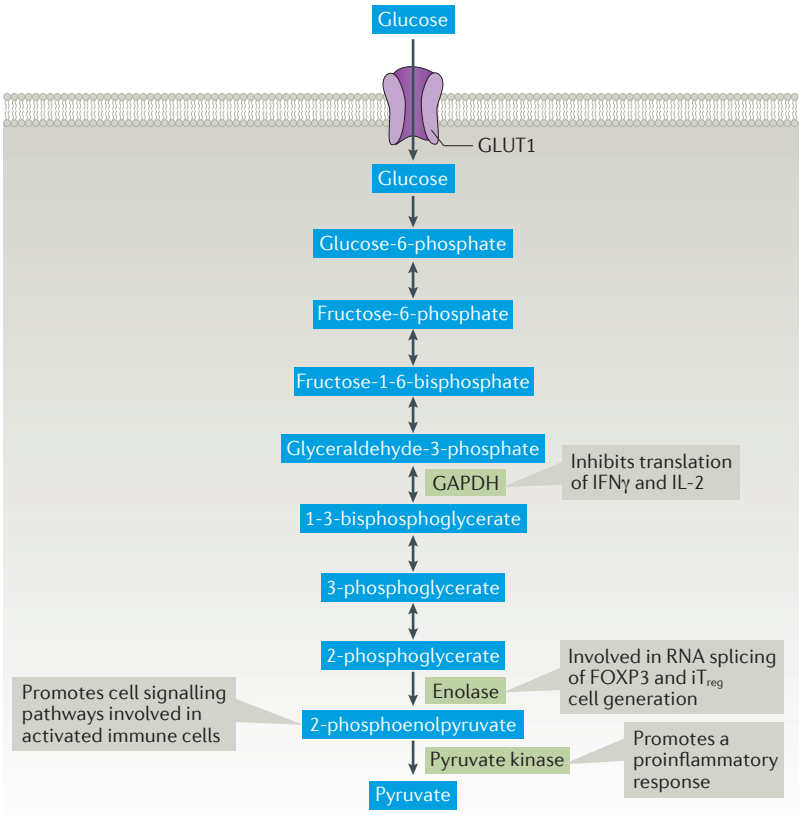


Figure 4 | **Immune regulatory roles of glycolytic intermediates.** Glycolytic intermediates not only influence immune function and inflammation by their role in metabolism, but also by specifically regulating various processes. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) also functions as an RNA-binding molecule that can inhibit the translation of *IFNG* and *IL2* mRNA. High glycolytic flux forces excess GAPDH into the glycolytic process, thereby relieving RNA from inhibition. Reduction of glycolytic flux releases enolase from the glycolytic pathway, upon which it enters the nucleus to aid the formation of the alternative splice variant of the transcription factor FOXP3, FOXP3-E2, generating potently immunosuppressive induced regulatory T (iT_{reg}) cells. The glycolytic intermediate 2-phosphoenolpyruvate promotes Ca^{2+} signalling, which supports T cell activation during high rates of glycolysis. When the M2 isoenzyme of pyruvate kinase (PKM2) is activated as a tetramer, it supports flux through glycolysis into the tricarboxylic acid cycle. As a dimer, PKM2 either acts as co-activator of hypoxia-inducible factor 1α, or it supports signal transduction by phosphorylating signal transducer and activator of transcription 3, which both support proinflammatory immune responses.

In 1995, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was discovered to directly bind to AU-rich regions of RNA, thereby inhibiting RNA translation[98]. In activated $CD4^+$ T cells that are provided with co-stimulation and growth factors but blocked from engaging glycolytic pathways, GAPDH also binds to the 3′ untranslated regions (3′UTRs) of *IFNG* and *IL2* mRNA; as a result, the ability of these cells to produce IFNγ is markedly compromised[99]. During aerobic glycolysis, GAPDH was utilized as a glycolytic enzyme, releasing *IFNG* and *IL2* mRNA for translation. Thus, modulation of GAPDH expression levels and regulation of glycolysis controlled effector cytokine production[99]. In a 2016 study, GAPDH was also found to bind to the AU-rich 3′UTR of *HIF1A* mRNA in glycolytically inactive naive and memory T cells, although in effector T cells with active glycolysis and decreased GAPDH availability, HIF-1α expression was elevated[100]. These data suggest that glycolytic metabolism is able to regulate the translation of *HIF1A* mRNA to enable T cells to adapt to hypoxia.

By contrast, during repression of glycolysis, a translational variant of the glycolytic enzyme enolase inhibits the formation of the alternative spliced variant FOXP3-E2 in potently anti-inflammatory induced T_{reg} (iT_{reg}) cells[101]. Pyruvate dehydrogenase (PDH) (which catalyses the conversion of cytosolic pyruvate into mitochondrial acetyl-CoA) and its inhibitor, PDH kinase 1 (PDHK1) (which promotes the glycolytic pathway), both also influence the balance between T_H17 cells and T_{reg} cells. Inhibition or knockdown of PDHK1 in mice increased the number of T_{reg} cells and diminished the number of T_H17 cells, resulting in protection against autoimmunity, owing in part to an accumulation of ROS[57].

Another glycolytic enzyme with a dual role is the M2 isoenzyme of pyruvate kinase (PKM2)[102]. Pyruvate kinase catalyses the conversion of 2-phosphoenolpyruvate and ADP into pyruvate and ATP during the final, rate-limiting step of glycolysis. PKM2 exists both as a dimeric enzyme and as a tetrameric enzyme. The tetrameric form is highly active in converting 2-phosphoenolpyruvate into pyruvate, and functions in a similar way to pyruvate kinase to provide pyruvate for the TCA cycle. By contrast, dimeric PKM2 is enzymatically almost inactive[102]. This less-active, dimeric form of PKM2 makes up the majority of cellular PKM2 and is strongly upregulated in LPS-activated macrophages, thereby supporting aerobic glycolysis and biosynthetic processes[23,103,104].

PKM2 also participates in non-glycolytic processes upon its translocation to the nucleus following mitogenic, oncogenic and LPS stimulation. In these non-glycolytic processes, PKM2 acts as a co-activator of HIF-1α, helping to promote the Warburg effect[105]. Briefly, upon LPS activation, PKM2 translocates to the nucleus where it forms a transcriptional complex with HIF-1α, binding directly to the *IL1B* promoter and initiating *IL1B* transcription[106]. In sepsis, PKM2 promotes the release of high mobility group box 1 (HMGB1), which acts as a potent proinflammatory cytokine, through its interaction with and activation of HIF-1α[107,108]. PKM2 that has translocated to the nucleus upon LPS stimulation either binds to the *STAT3* promoter, or phosphorylates STAT3,

enhancing the transcription and subsequent activation of STAT3 and boosting the expression of IL-1β and IL-6 (REFS 104,109). In mast cells, PKM2 interacts with ITAM motifs on the γ-chain of the high-affinity IgE receptor, resulting in a decrease in PKM2 activity, which is essential for mast cell degranulation[110].

PKM2 is not only a key enzyme in glycolysis, but is also important for several inflammatory processes. It is therefore not surprising that many, if not all, inflammatory disorders are associated with increased expression of PKM2 (REFS 99,111–114).

Metabolites that control inflammation

Not only do metabolic programmes differ between immune cell populations and according to immune cell functions, they also result in different types of metabolites being produced in different amounts, which, in turn, can directly affect immune cell responses. For example, the glycolytic intermediate 2-phosphoenolpyruvate, levels of which increase during glycolysis, inhibits the re-uptake of Ca^{2+} into the endoplasmic reticulum, thereby sustaining levels of Ca^{2+} (an important second messenger) in the cytoplasm and promoting cell signalling via the transcription factor NFAT1, which is involved in T cell activation[115] (FIG. 4).

In M1 macrophages, interruption of the TCA cycle results in a massive accumulation of TCA intermediates such as α-ketoglutarate, fumarate and succinate[52]. The metabolites α-ketoglutarate, fumarate and succinate regulate the activity of hypoxia-inducible factors by inhibiting the activity of hydroxylases[72,116–118]. As a result of this inhibition, HIF-1 upregulates a variety of proinflammatory molecules (such as IL-1β) and anti-inflammatory molecules (such as the microRNA miR-210 or programmed cell death 1 ligand 1) in myeloid cells[72,119–122].

Moreover, TCA intermediates participate in the epigenetic control of gene expression as important substrates or inhibitors for DNA-modifying and histone-modifying enzymes (FIG. 5). For example, the enzymes of the TET family (which facilitate DNA demethylation) and the JmjC-domain-containing histone demethylases both require α-ketoglutarate as a substrate and are repressed by succinate[123]. Epigenetic modulation by methylcytosine dioxygenase TET2 affects not only T_H1 cell and T_H17 cell cytokine responses[124], but also the stability of FOXP3 expression in iT_{reg} cells[125]. Lysine-specific demethylase 6B has a critical role in the regulation of $CD4^+$ T cell differentiation[126], and is associated with gene expression changes in LPS-stimulated macrophages[127].

Another epigenetic modification that affects DNA structure and gene expression is the acetylation and deacetylation of histones. Acetylation of histones by histone acetyltransferases requires acetyl-CoA, which is supplied via various mechanisms such as metabolism of the TCA intermediate citrate or by enhanced lactate dehydrogenase expression or activity. The latter mechanism creates high concentrations of acetyl-CoA, resulting in increased histone acetylation and subsequent transcription of *IFNG*, thus promoting effector T cell differentiation[128]. Deacetylation of histones by sirtuins is directly connected to cellular metabolism, as it is regulated by the balance of oxidized NAD^+ and reduced NADH[129]. Sirtuins use oxidized NAD^+ as a substrate for the deacetylation process and are inhibited by its reduced form. Sirtuins do not solely modulate histone deacetylation; they are also capable of modulating immune responses directly by deacetylating FOXP3 (thereby inhibiting T_{reg} cell responses)[130,131], by deacetylating the transcription factor RORγt (thereby promoting T_H17 cell responses)[132], or by reducing inflammatory responses through inhibition of the transcription factor NF-κB[133]. Moreover, sirtuins are associated with the regulation of so-called clock genes, thus providing a direct link between immunometabolism and circadian rhythms[134,135].

In addition, the metabolic shift in glucose metabolism from oxidative phosphorylation to aerobic glycolysis (which leads to an increase in TCA intermediates and skews the NAD^+:NADH ratio) provides nonspecific innate immune protection from recurrent infections, aiding the development of memory characteristics by the innate immune system (also known as trained immunity)[129,136].

Key regulators of immunometabolism

The majority of immune cells participating in an inflammatory reaction (such as activated M1 macrophages and T_H17 cells) shift their metabolism towards enhanced glucose uptake, aerobic glycolysis and increased activity of the PPP and fatty acid synthesis. Key regulators of these changes are mTOR and the transcription factors Myc and HIF-1α, which oppose AMP-activated protein kinase (AMPK) in these processes[82]. mTOR activation supports cell growth, proliferation and effector function by sensing amino acids and growth factors and by upregulating mRNA translation and lipid synthesis[48,137]. Moreover, mTOR helps to maintain cellular and nuclear levels of Myc, which induces a glycolytic gene expression profile[42,45,46]. Thus, mTOR activation supports both the differentiation of T cells and monocytes into proinflammatory T cell subsets and M1 macrophages, respectively, and the proinflammatory effector functions of these cells[48,137]. Interestingly, the type of mTOR complex used during glycolytic reprogramming of T cells upon activation differs depending on the differentiation state of the T cell[37]. The induction of aerobic glycolysis in proliferating effector T cells after primary antigen challenge requires mTORC1 signalling, whereas immediate–early glycolytic reprogramming after a recall response in memory T cells needs mTORC2 signalling[37]. By contrast, immune cells with low rates of glycolysis and high oxidative metabolism tend to be long-lived with anti-inflammatory and regulatory properties, such as M2 macrophages, T_{reg} cells and quiescent memory T cells. Thus, reduction of glycolysis by inhibition or deletion of mTOR complexes in T cell receptor (TCR)-activated T_H cells supports the development of T_{reg} cells while preventing the generation of effector T cell subpopulations[47,138].

In opposition to mTOR, AMPK promotes anti-inflammatory and regulatory properties in immune cells and limits effector cell responses, thereby inhibiting mTOR activity[82]. AMPK is activated by low levels of cellular energy (for example, a high AMP:ATP ratio) and during nutrient deprivation, resulting in a catabolic

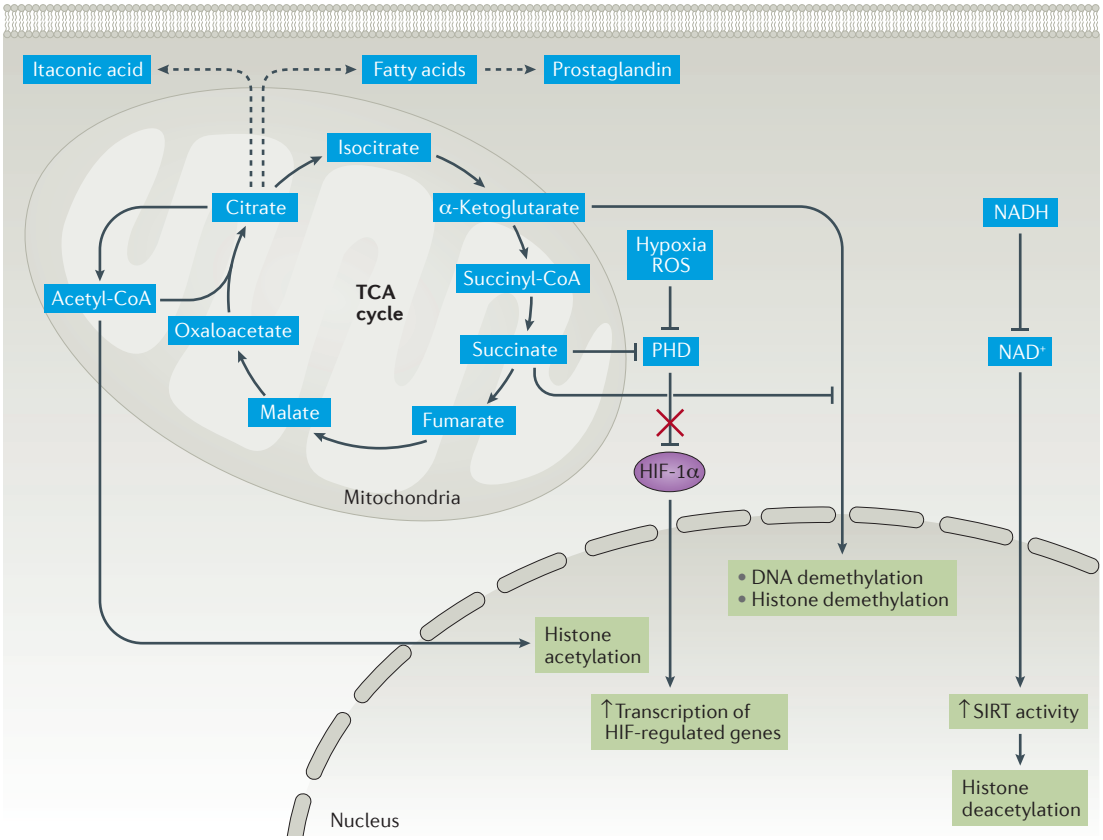


Figure 5 | **Regulation of inflammation by metabolites.** Citrate serves as a substrate for the antimicrobial molecule itaconic acid and is necessary for the production of prostaglandin. Accumulation of the tricarboxylic acid (TCA) cycle intermediate succinate leads to inhibition of prolyl hydroxylases (PHDs), negative regulators of hypoxia-inducible factor 1α (HIF-1α), thereby promoting HIF-mediated gene transcription, which enhances glycolysis and both proinflammatory and anti-inflammatory immune responses. Cellular metabolism is also critically involved in epigenetic processes. Acetyl-CoA is a key substrate for histone acetylation, whereas histone deacetylation by sirtuins (SIRT) is aided by a high ratio of NAD^+ to NADH. Moreover, a high ratio of α-ketoglutarate to succinate promotes the demethylation of DNA and histones, thereby linking cellular metabolism to epigenetic control of immune responses. ROS, reactive oxygen species.

turnover of molecules such as fatty acids[82]. In T_{reg} cells, memory T cells and M2 macrophages, AMPK and TNF receptor-associated factor 6 support oxidative metabolism[17,22,137,139,140].

Glycolytic reprogramming of effector T cells and M1 macrophages requires transcriptional changes mediated by a variety of transcription factors. These changes include the activation of HIF-1 and Myc and a reduction in levels of the transcription factor BCL6, which competes with both HIF-1α and Myc to bind to DNA in T_H1 cells, and result in the repression of glycolytic reconfiguration[36,45,120,129]. Myc is essential for the initial metabolic switch to glycolysis, but its expression is transient and decreases after activation. Therefore, in $CD8^+$ T cells, the Warburg effect is maintained by the transcription factors AP-4 and IRF4, downstream targets of Myc[141,142]. AP-4 induces a similar glycolytic transcriptional profile to Myc, supporting aerobic glycolysis and effector function in $CD8^+$ T cells[141]. IRF4 also cooperates with Myc to sustain aerobic glycolysis in a later phase of $CD8^+$ T cell activation[142].

Immunometabolism in rheumatic diseases

Alterations of metabolic configurations of immune cells can contribute to dysfunctional immune responses. For instance, a dysfunctional T cell response is a typical feature of autoimmunity and is associated with altered metabolic cellular configurations. Naive $CD4^+$ T cells from patients with RA should immediately induce the Warburg effect following TCR engagement, but instead are characterized by low levels of ATP and lactate compared with T cells from healthy individuals[10]. Due to decreased expression of PFKFB3, T cells from patients with RA shunt glucose towards the PPP (a process termed the 'anti-Warburg effect'), resulting in the accumulation of NADPH and a reduced intracellular level of ROS[10]. Restoring intracellular ROS was able to correct the abnormal proliferative behaviour of T cells from patients with RA and to suppress synovial inflammation in a human synovium-NSG chimeric mouse model[143]. In a study published in 2016, diminished expression of PFKFB3 in healthy individuals at risk of developing RA and individuals

with established RA was associated with impaired induction of tyrosine-protein phosphatase non-receptor type 22 (PTPN22)[144]. However, further investigation is required to elucidate the reasons behind the failure of PFKFB3 and PTPN22 induction in T cells from patients with RA.

In contrast to T cells from patients with RA, glycolysis and mitochondrial oxidative metabolism are activated in T_H cells from patients with SLE and in T cells from lupus-prone mice that have concomitant mTORC1 activation[3,9]. The high levels of glucose and oxygen consumption seen in T_H cells from patients with SLE were also detected in naive T cells, and were not a result of immune activation, suggesting an intrinsic malfunction in cells from these patients[3]. Interestingly, T_H cells from patients with RA and patients with SLE have different metabolic dysfunctions. T_H cells from patients with RA are characterized by reduced glycolytic flux, leading to energy deprivation, whereas T cells from patients with SLE are metabolically overactive, leading to excessive ROS production[3,9,10,143]. For a more detailed and comprehensive view of the metabolic configuration of immune cells in SLE and RA, see the Reviews by Morel[11] and Weyand & Goronzy[12] in this journal.

Although the metabolic configuration of T cells is seemingly different in RA and SLE, these disorders, as well as other chronic rheumatic diseases (such as ankylosing spondylitis, systemic sclerosis and idiopathic inflammatory myopathies) have been associated with an increased risk of coronary artery disease in large epidemiological studies[145–150]. A study published in 2016 found that the metabolic configuration of monocytes and macrophages from patients with coronary artery disease seem to be unbalanced, leading to oxidative stress and tissue inflammation[104]. Whether a generally unbalanced metabolism in monocytes from patients with rheumatic diseases might contribute to cardiovascular comorbidities remains to be clarified.

Conclusions

Cellular metabolism is considerably varied and has dual roles in immune cells: the delivery of energy and substrates for biosynthesis and the direct regulation of immune cell function. During inflammation, alterations in the tissue microenvironment, characterized by hypoxia and competition for nutrients with high numbers of infiltrating cells, force immune cells to adapt by reprogramming their metabolism. Metabolic reconfiguration varies between innate and adaptive immune responses, and between effector and resolution phases depending on a cell's specific functional needs. These metabolic changes are not only a response to an inflammatory microenvironment; they also guide immune cell differentiation and function via several mechanisms, including the differential activities of metabolic enzymes, metabolites and metabolic intermediates and the activity of key regulators of metabolism such as HIF-1. Failure to reprogramme metabolism ultimately results in a dysregulated immune response, as seen in rheumatic diseases.

Increasing our knowledge of immune cell metabolism will, it is hoped, create numerous opportunities for the development of novel therapeutic strategies to modulate these reprogramming mechanisms by specifically targeting metabolic intermediates or molecules at metabolic checkpoints. Although several existing immunosuppressant drugs (such as methotrexate) are known to interfere with cell metabolism by directly or indirectly targeting key metabolic checkpoints, increasing our knowledge of their mechanisms of action during metabolic reprogramming in inflammation will be invaluable for developing new therapeutic strategies. For instance, combined treatment with immunosuppressive therapies and drugs that directly target cellular metabolism might increase the pharmacological efficacy and reduce the adverse effects of classic immunosuppressive drugs. Clearly, more research is needed to increase our knowledge of the biochemistry of immune cells and its effect on immune responses.

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Author contributions

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REVIEWS

Immunometabolism in systemic lupus erythematosus

Laurence Morel

Abstract | Systemic lupus erythematosus (SLE) is an autoimmune disease mediated by pathogenic autoantibodies directed against nucleoprotein complexes. Beyond the activation of autoreactive B cells, this process involves dysregulation in many other types of immune cells, including $CD4^+$ T cells, dendritic cells, macrophages and neutrophils. Metabolic substrate utilization and integration of cues from energy sensors are critical checkpoints of effector functions in the immune system, with common as well as cell-specific programmes. Patients with SLE and lupus-prone mice present with activated metabolism of $CD4^+$ T cells, and the use of metabolic inhibitors to normalize these features is associated with therapeutic effects. Far less is known about the metabolic requirements of B cells and myeloid cells in SLE. This article reviews current knowledge of the alterations in metabolism of immune cells in patients with SLE and mouse models of lupus in the context of what is known about the metabolic regulation of these cells during normal immune responses. How these alterations might contribute to lupus pathogenesis and how they can be targeted therapeutically are also discussed.

Systemic lupus erythematosus (SLE) is an autoimmune disease with a heterogeneous clinical presentation that results from a wide array of immunological abnormalities. Tissue damage is mediated by pathogenic autoantibodies, but abnormalities in cell development and function are not limited to B cells; indeed, most types of immune cell have been implicated in SLE pathogenesis[1]. A number of spontaneous and induced mouse models of lupus have been developed[2], each corresponding to overlapping subsets of the clinical and immunological phenotypes presented by patients with SLE (BOX 1).

Immune cells respond to stimuli with a rapid proliferation and differentiation into highly specialized effector cells. These processes are metabolically demanding, requiring not only an increased uptake of nutrients (mostly glucose, fatty acids and glutamine), but also a switch to specialized metabolic pathways corresponding to specific effector functions. The changes in the amount and type of nutrients taken up, as well as the metabolic processes by which they are utilized, have been referred to as metabolic reprogramming. Immunometabolism refers to the metabolic reprogramming that occurs during immune responses. Metabolic abnormalities in T cells from patients with SLE were first reported 15 years ago[3], and these observations provided the rationale for small clinical trials to explore whether reducing mechanistic target of rapamycin (mTOR) activation by use of rapamycin or the glutathione precursor N-acetylcysteine could be a promising therapeutic approach[4,5]. This Review discusses these findings, as well as subsequent studies in patients with SLE and in mouse models of lupus, in the context of recent discoveries that have placed metabolism as a central checkpoint for effector programmes first in T cells, and increasingly in other immune cell types.

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T cell metabolism in SLE

A functional link between metabolism and immune cell function was first discovered in T cells[6]. $CD4^+$ T cells in patients with SLE and lupus-prone mice have altered signalling and function[7], and numerous abnormalities in the metabolism of these cells have been reviewed elsewhere[8–10]. Only salient points are summarized and integrated here (FIG. 1).

Mitochondrial abnormalities and oxidative stress. A dysfunction in cellular metabolism in SLE was first reported in T cell mitochondria, which are characterized by membrane hyperpolarization (elevation of the mitochondrial transmembrane potential), increased production of reactive oxygen intermediates and ATP depletion[11]. This pioneering study also documented depletion of the intracellular antioxidant glutathione (in its reduced state) in SLE[11]. The increased mitochondrial mass ('megamitochondria') and hyperpolarization of mitochondria in $CD4^+$ T cells from patients with SLE contribute to the activation, impaired activation-induced cell death and increased necrosis of these cells, with

Key points

- In systemic lupus erythematosus (SLE), $CD4^+$ T cells have a hypermetabolic state dominated by oxidation, mitochondrial abnormalities, activation of mTORC1 and increased glucose flux
- Targeting T cell metabolism has therapeutic effects in mouse models of lupus and in the T cells of patients with SLE
- Cell-specific metabolic imbalances probably also affect other immune cells in SLE, including neutrophils, plasma cells and macrophages, and specific metabolic targeting of these cells could have therapeutic benefit
- A better understanding of the complexities of immunometabolism in SLE could lead to personalized therapeutic options
- The metabolome, potentially intersecting with the microbiota, might provide biomarkers for SLE

necrosis adding to the pool of cellular debris that stimulates autoreactive lymphocytes[12]. Increased mitochondrial biogenesis in response to elevated nitric oxide (NO) levels, combined with defective mitophagy, are the major factors responsible for the increased mitochondrial mass and size of $CD4^+$ T cells in SLE[13]. Mitochondrial remodelling directly controls metabolic reprogramming of T cells. Mitochondrial fusion promotes assembly of the electron transport chain and thus sustains oxidative phosphorylation, which is associated with T cell memory and longevity. Conversely, mitochondrial fission disrupts the electron transport chain and forces a switch to aerobic glycolysis, which is associated with acute T cell activation[14]. These findings are in agreement with the large mitochondrial size and increased oxidative phosphorylation in $CD4^+$ T cells in SLE[15]. Despite hyperactivity of electron transport chain complex I, ATP production is decreased in these T cells, leading to the release of reactive oxygen intermediates[16]. This oxidative stress can promote autoimmunity by modulating signal transduction and cytokine production in a T cell-intrinsic manner[17]. A well-documented consequence of oxidative stress in T cells in SLE is the lysosomal degradation of the T cell surface glycoprotein CD3ζ chain and its replacement by the highly homologous FcεRIγ chain, which recruits tyrosine-protein kinase SYK and enhances signalling upon T cell receptor (TCR) activation[18]. Treatment with N-acetylcysteine replenished the levels of glutathione and, indirectly, NADPH, which represent two major components of the defence system against oxidative stress in the mitochondria. This treatment showed a therapeutic effect in patients with SLE[5], demonstrating a pathogenic consequence of mitochondrial dysfunction and oxidative stress in T cells in SLE.

Genetic factors might contribute to mitochondrial dysfunction in T cells in SLE. In mice, the *Sle1c2* lupus susceptibility locus is associated with increased $CD4^+$ T cell activation, age-dependent expansion of IFNγ-producing T cells and decreased regulatory T (T_{reg}) cell frequency[19]. T cells expressing *Sle1c2* have increased mitochondrial mass and express a reduced level of *ESRRG*, a gene that regulates mitochondrial metabolism[20]. A polymorphism in *MT-ATP6*, which encodes a component of mitochondrial membrane ATP synthase (complex V or F_0F_1 ATP synthase), is associated with SLE[21] and might be one of the factors contributing to the decreased ATP levels found in T cells in SLE. A polymorphism in *UCP2* is also associated with SLE susceptibility[22]. *UCP2* encodes a protein that uncouples protons from ATP synthesis, thereby negatively regulating mitochondrial reactive oxygen species (ROS) production, and potentially contributing to the oxidative state observed in T cells in SLE. However, the same polymorphism is also associated with susceptibility to rheumatoid arthritis (RA), and T cells in RA are characterized by a hyper-reduced state[23], making the consequences of this association unclear.

mTOR. Mechanistic target of rapamycin (mTOR) is a sensor system composed of two complexes, mTOR complex 1 (mTORC1) and mTORC2, which integrate metabolic cues to direct genetic programmes regulating cellular growth and energy utilization. Polarization of T cells to the inflammatory type 1 T helper (T_H1) and T_H17 subsets is mTORC1-dependent in both normal[24,25] and autoimmune[26] T cells. Conversely, inhibition of mTORC1 expands T_{reg} cells[26], although mTORC1 is required for the suppressive function of these cells[27]. The requirement for mTOR in $CD4^+$ follicular helper T (T_{FH}) cells, a critical subset in patients with SLE and in lupus-prone mice[28,29], is unclear. Virally induced T_{FH} cells do not require mTORC1 activation to the same extent as their T_H1 cell counterparts[30]. However, inhibition of AMP-activated protein kinase (AMPK) and subsequent activation of mTORC1 by Roquin-1 promotes T_{FH} cell differentiation[31] and a lupus-like phenotype[32]. Finally, emerging results suggest a context-dependent regulation of T_{FH} cells by mTORC1 or mTORC2 in the normal immune response in mice[33]. The role of mTOR in T_{FH} cell polarization and function remains to be addressed in SLE in patients as well as in mouse models of spontaneous lupus.

mTORC1 activation in $CD4^+$ T cells from patients with SLE[34] and lupus-prone mice[35–37] has multiple consequences; most importantly, it enhances glycolysis and prevents autophagy, including mitophagy, which is likely to contribute to mitochondrial dysfunction. This dysfunction leads to the development of pathogenic $CD4^+$ T cell phenotypes in patients with SLE[38,39], which is reverted by treatment with rapamycin or N-acetylcysteine (which inhibits mTORC1)[4,5], including the reduction of CD3ζ degradation and normalization of TCR signalling[40]. Several overlapping mechanisms lead to mTORC1 activation in T cells in SLE, including mitochondrial dysfunction, over-reactivity of the pentose phosphate pathway (PPP) and transaldolase activity[40], as well as accumulation of kynurenine, a tryptophan metabolite with immune modulatory functions[41]. Genetic activation of mTORC1 is also associated with SLE. The development of severe lupus-like pathology has been reported in four patients suffering from tuberous sclerosis[42–45], a rare disease resulting from mutations in the genes encoding hamartin (*TSC1*) or tuberin (*TSC2*), which together form the TSC complex that inhibits mTORC1 activation. Given that the coincidence of tuberous sclerosis and SLE is statistically unlikely, these case reports illustrate the link between unrestrained mTORC1 activation and the development of SLE.

Electron transport chain
A series of proteins in the inner mitochondrial membrane that transfer electrons from one to the other in a series of redox reactions, resulting in the movement of protons out of the mitochondrial matrix and in the synthesis of ATP.

Oxidative phosphorylation
A metabolic pathway that produces ATP from the oxidation of acetyl-CoA and the transfer of electrons to the electron transport chain via NADH and $FADH_2$.

Aerobic glycolysis
(Also known as the 'Warburg effect') The abrupt metabolic switch from oxidative phosphorylation to glycolysis, regardless of the availability of oxygen, to provide energy for cell proliferation and effector functions.

Glycolysis
An oxygen-independent metabolic pathway that generates two molecules of pyruvate, ATP and NADH from every one molecule of glucose, supporting the tricarboxylic acid cycle and providing intermediates for the pentose phosphate pathway, glycosylation reactions and for the synthesis of biomolecules (including serine, glycine, alanine and acetyl-CoA).

Box 1 | **Major manifestations in SLE and mouse models**

Clinical manifestations and immune abnormalities in SLE

According to the Systemic Lupus International Collaborating Clinics (SLICC) scheme for the classification of SLE, a patient must present with at least four criteria from among 11 clinical criteria and six immunologic criteria, including at least one clinical and at least one immunologic criterion, or with biopsy-proven nephritis and anti-nuclear antibodies (ANA) or anti-double-stranded DNA (anti-dsDNA) antibodies[140].

Corresponding phenotypes in mouse models

The incidence of SLE is heavily gender-biased with a 9:1 female:male ratio. This female preponderance is also found in all mouse models of lupus to varying degrees, except in the BXSB.Yaa mouse, in which disease is driven by a translocation of X-chromosome encoded *Tlr7* to the Y chromosome. All spontaneous mouse models are characterized by the production of ANA and anti-dsDNA antibodies, as well as the development of immune complex glomerulonephritis. Antibodies with RNA specificities (including anti-Sm antibodies) as well as antiphospholipid antibodies are found in a subset of strains; the former are found in MRL/lpr and BXSB.Yaa mice, and the latter in MRL/lpr and (NZW×BXSB)F1 mice. Although type I interferon has a role in spontaneous mouse models of lupus, the interferon signature is not as dominant in these models as in patients with SLE or in the pristane-induced mouse model. Besides renal pathology, MRL/lpr mice present with skin lesions that have most features of cutaneous lupus, some cognitive impairment that represents a subset of neuropsychiatric lupus manifestations, as well as low incidence of joint inflammation.

SLE, systemic lupus erythematosus.

Glucose metabolism. Three main pathways of glucose utilization — PPP, oxidative phosphorylation and aerobic glycolysis — have been implicated in T cell activation in SLE. PPP hyperactivation corresponds to the metabolic needs of proliferating T cells during the development of effector responses, and produces a strong metabolic signature in the peripheral blood lymphocytes of patients with SLE[41]. Whether T cell activation in SLE is the primary cause of PPP hyperactivation or, alternatively, accelerated PPP enables T cell activation is unclear. Interestingly, PPP is also hyperactivated in the T cells of patients with RA, in which the resultant generation of excess NADPH is responsible for the inflammatory T cell phenotype[23]. The reasons for these opposite outcomes of PPP activation in T cells in SLE and RA are presently unclear.

Chronically activated healthy human $CD4^+$ T cells[46], as well as $CD4^+$ T cells from patients with SLE[15,35] and lupus-prone mice[35,36], showed high levels of oxygen consumption, in contrast to acutely activated T cells, which had a more glycolytic phenotype[46]. These findings suggest that chronic activation by autoantigens (as occurs in SLE) is supported by oxidative phosphorylation, whereas aerobic glycolysis supports acute activation induced by foreign antigens or *in vitro* supraphysiological TCR stimulation. $CD4^+$ T cells from patients with SLE and lupus-prone mice also display elevated glycolysis[35,36], which could correspond to a compensatory mechanism to remediate ATP production by defective mitochondria. A dual requirement for glycolysis and oxidative phosphorylation has been found in healthy effector memory $CD4^+$ T cells[47], a subset that is expanded in SLE[48,49]. The enhanced glycolysis and oxidative phosphorylation found in naive $CD4^+$ T cells from lupus-prone mice[35] suggests that T cells in SLE might be fuelled by an intrinsically high metabolism, and that the age-dependent accumulation of effector memory $CD4^+$ T cells further increases T cell metabolism.

Several glucose transporters are expressed on T cells, with GLUT1 and GLUT6 being highly upregulated upon TCR and CD28 stimulation. Increased expression of glucose transporters leads to increased glucose uptake and glycolysis[50]. *Glut1* overexpression in mice led to the accumulation of activated T cells and the production of autoantibodies[51]. *GLUT1* overexpression has not been reported in human SLE, but $CD4^+$ T cells from lupus-prone B6 Sle1.Sle2.Sle3 triple-congenic mice overexpressed two key genes in glycolysis[35,36]: *Slc16a3*, which encodes a lactate transporter, and *Hif1a*, encoding a transcription factor induced by mTORC1 activation[25]. It is therefore likely that *HIF1A* is upregulated in T cells from patients with SLE. Hypoxia-inducible factor 1α (HIF-1α) regulates T cell effector functions[52]; the role of *HIF1a* in SLE is discussed further in the hypoxia section of this Review. A link between the complement receptor CD46 (also known as membrane cofactor protein), which is activated in T cells in SLE[53], *Glut1* expression and mTORC1 activation has been found in mice[54]. Dysregulation of complement signalling in T cells might therefore contribute to the metabolic abnormalities of T cells in SLE.

Cholesterol and glycosphingolipid metabolism. Glycosphingolipids and cholesterol are inserted in lipid rafts in the T cell plasma membrane and are important in regulating TCR signalling. In normal T cells, levels of glycosphingolipids and cholesterol increase in these rafts following TCR activation, then return to baseline once TCR signalling ceases[55]. Lipid rafts are aggregated in $CD4^+$ T cells from patients with SLE[56]. Reduction of lipid raft synthesis restored normal signalling in T cells from patients with SLE[57,58] and decreased lupus pathology in MRL/lpr mice[59].

Studies published in 2016 unveiled a critical role for cholesterol in regulating TCR signalling and effector functions in $CD8^+$ T cells[60]. Displacing cholesterol with cholesterol sulfate (a naturally occurring cholesterol analogue) prevented TCR clustering and decreased signalling[61]. On the other hand, biochemical studies showed that cholesterol binds TCRβ and prevents its phosphorylation[62]. As yet, these observations have not been extended to autoreactive T cells. However, *in vitro* polarized mouse T_H17 cells are characterized by increased cholesterol uptake and biosynthesis coupled with decreased metabolism and efflux, leading to the production of specific sterol–sulfate conjugates that activate nuclear receptor RORγ[63]. In addition, statins have been used to treat SLE in clinical trials and preclinical animal models, with the results showing the expected reduction in cardiovascular morbidity, but mixed results with respect to the non-cardiovascular pathology associated with the disease[64]. Statins prevented T_H17 polarization in patients with multiple sclerosis and promoted T_{reg} cell expansion in patients with RA (reviewed by Ulivieri and Baldari[64]); this observation suggests that statins could be beneficial for patients with SLE, given the role of these T cell subsets in the disease[7]. However, the fact that statins not only inhibit cholesterol synthesis, but also have immunoregulatory effects by preventing protein isoprenylation, makes it difficult to interpret these results from a mechanistic perspective.

Pentose phosphate pathway (PPP)
An anabolic metabolic pathway parallel to glycolysis that branches out from glycolysis with the conversion of glucose-6-phosphate to ribose 5-phosphate and generates the reducing equivalents NADPH, ribose 5-phosphate (used in the synthesis of nucleotides and nucleic acids) and erythrose-4-phosphate (used in the synthesis of amino acids).

Lipid rafts
Microdomains of the plasma membrane that are enriched in cholesterol and glycosphingolipids and serve as self-organizing centres for the assembly of signalling molecules.

Statins
A class of lipid-lowering drugs that inhibit a key enzyme in the synthesis of cholesterol, HMG-CoA reductase.

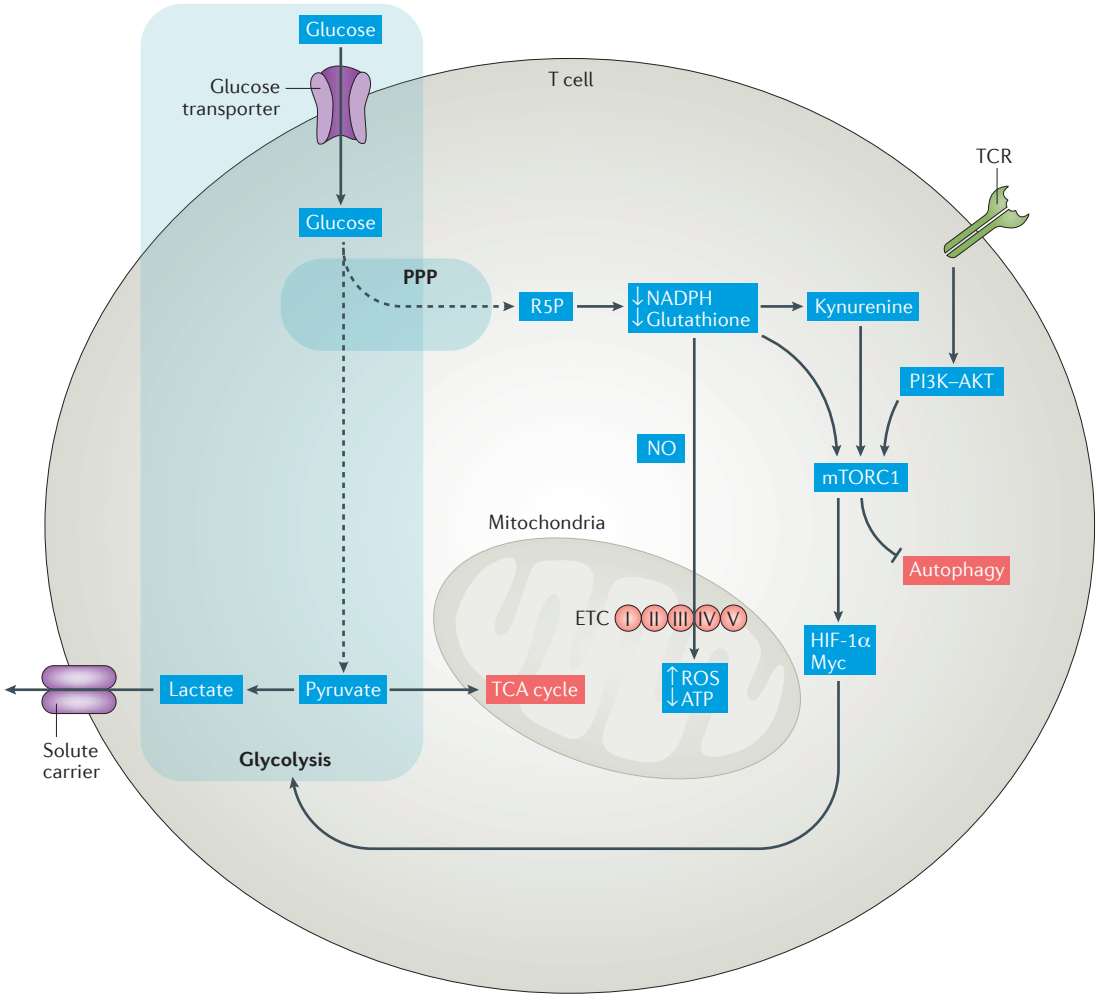

Figure 1 | **Metabolic activation of CD4⁺ T cells in SLE.** Glucose is mostly oxidized in the tricarboxylic acid (TCA) cycle but can also be diverted to the pentose phosphate pathway (PPP). T cell receptor (TCR) stimulation activates mechanistic target of rapamycin complex 1 (mTORC1) through the phosphatidylinositol 3-kinase (PI3K)–AKT pathway. Low levels of NADPH and glutathione contribute to elevated production of mitochondrial reactive oxygen species (ROS) but decreased levels of ATP. Low NADPH and glutathione also contribute to mTORC1 activation, directly and/or through elevated levels of kynurenine. mTORC1 activation activates glucose metabolism through hypoxia-inducible factor 1α (HIF-1α) and Myc proto-oncogene protein, and inhibits autophagy, which itself contributes to mitochondrial dysfunction. ETC, electron transport chain; NO, nitric oxide; R5P. ribose 5-phosphate.

T cells from patients with SLE support increased glycosphingolipid synthesis, which is associated with increased TCR signalling[58]. Pharmacological inhibition of glycosphingolipid synthesis reduced the activation of these T cells *in vitro*[58], demonstrating a causal relationship. Several mechanisms could be responsible for increased glycosphingolipid levels in T cells in SLE. Increased expression of oxysterols receptor LXRβ (also known as liver X receptor β), a nuclear receptor that controls cellular lipid metabolism and trafficking, was associated with altered trafficking and recycling of glycosphingolipids, leading to their accumulation in T cells from patients with SLE[58]. The role of LXRs in immune cells is complex, however, involving both proinflammatory and anti-inflammatory functions[65]. A genetic polymorphism leading to decreased LXRα levels in B cells and increased B cell proliferation has been associated with SLE susceptibility[66]. Deficiency in both the LXRα and LXRβ genes promotes *IL17* transcription by aryl hydrocarbon receptor, indicating that LXRs have immunoregulatory functions unrelated to cholesterol homeostasis[67]. Another potential contributor to glycosphingolipid synthesis in T cells in SLE is FLI1, a transcription factor expressed in T cells with expression levels linked to SLE pathogenesis[68]. One target of FLI1 is *NEU1*, which controls glycosphingolipid synthesis. Accordingly, *Fli1*-haplodeficiency reduced disease severity in MRL/lpr mice in parallel with decreased T cell activation[68]. The latter effect included reduced expression of CXC chemokine receptor 3 (CXCR3) by T cells and a correspondingly reduced level of renal infiltrates[69]. A variant in the *FLI1* promoter region that leads to increased *FLI1* expression is associated with susceptibility to SLE[70]. Glycosphingolipid metabolism is also defective in the kidneys of MRL/lpr mice and patients with SLE owing to increased expression of two enzymes, β-1,4-galactosyltransferase 5 (also known as β4GalT-5)

and neuraminidase 1 (encoded by *NEU1*), involved in glycosphingolipid biosynthesis[71]. Elevated glycosphingolipid levels have been associated with inflammation, including lupus nephritis[71], indicating that altered glycosphingolipid metabolism can affect SLE pathogenesis not only through the immune system, but also at the site of tissue injury.

Abnormal metabolism in other immune cells

B cells. Similar to $CD4^+$ T cells, activated B cells are predominantly glycolytic[72], but the existence of metabolic checkpoints in B cell development is poorly characterized. A few studies are, however, relevant to B cells in SLE. Transgenic mice overexpressing B cell activating factor (BAFF, also known as BLyS or TNF ligand superfamily member 13B) produce lupus-like autoantibodies and B cells from these mice are more glycolytic than B cells from nontransgenic littermates[73]. According to this result, B cells from patients with SLE and lupus-prone mice, which are exposed to high levels of BAFF, should also be highly glycolytic (FIG. 2), but this hypothesis has not been formally tested. Massive protein synthesis corresponding to immunoglobulin secretion coupled with longevity places unique metabolic demands on long-lived plasma cells[74]. Enforced mTORC1 activation drives B cells to differentiate into plasma cells[75], an effect that can be explained by the role of mTORC1 in promoting protein synthesis. mTORC1 is activated in T cells from patients with SLE[34] and in the B cells of lupus-prone mice in several models of disease[76]. *In vitro* studies have also shown that rapamycin inhibits BAFF-mediated proliferation and survival signals[77]. It is therefore likely that mTORC1 activation contributes to plasma cell differentiation and production of pathogenic autoantibodies in SLE. The metabolic requirements of long-lived plasma cells corresponding to their longevity was addressed in a 2016 study[78]. As shown previously for memory T cells[47], survival of long-lived plasma cells requires mitochondrial pyruvate import via the mitochondrial pyruvate carrier (MCP) complex[78]. Inhibition of glucose utilization could therefore target plasma cells in SLE, especially long-lived plasma cells, not only by preventing immunoglobulin glycosylation, but also by impairing their survival through lack of pyruvate. The latter effect should also be achieved by the use of MCP inhibitors, which could also impair the function of effector memory $CD4^+$ T cells in SLE[36].

The metabolic gene *Faah*, encoding fatty acid amide hydrolase (FAAH), has been proposed as a susceptibility gene in the murine NZM2410-derived *Sle2* locus[79]. FAAH degrades ligands for cannabinoid receptors and members of the peroxisome proliferator-activated receptor (PPAR) family. The lupus-associated allele corresponds to increased *Faah* expression and enhanced B cell receptor revision in mature B cells, which leads to autoantibody production[79]. Interestingly, increased FAAH levels were found in plasma cells from patients with SLE[80]. The mechanism linking increased levels of fatty acid amides and esters to the B cell receptor repertoire or plasma cell function is unclear. Increased FAAH expression predicts decreased PPAR activation and, interestingly, multiple studies have shown a beneficial effect of PPARγ agonists in mouse models of lupus[81–84], potentially linking FAAH and PPARγ to SLE pathogenesis.

Macrophages and dendritic cells. Our understanding of the metabolic programmes that govern macrophage and dendritic cell (DC) functions has expanded rapidly in the past 5 years[85]. However, whether changes in the metabolism of these cells has a role in the pathogenesis of SLE is largely unknown. The tolerogenic clearance of apoptotic cells by splenic marginal zone macrophages requires the expression of indoleamine 2,3-dioxygenase (IDO)[86]. A primary downstream effector of IDO is the metabolic-stress sensing kinase eIF-2α-kinase GCN2, the activity of which is required to prevent autoimmunity induced by exposure to apoptotic cells[87]. In response to amino acid variations such as tryptophan catabolism by IDO, GCN2 activates a stress-response programme that regulates innate immunity[88]. The protective effect of GCN2 against autoimmune pathology has also been reported in kidneys of mice with immune complex-induced nephritis[87]. IDO and GCN2 expression protected podocytes from apoptosis and reduced glomerular infiltrates by a process involving autophagy, following immune complex-induced inflammation. Although the molecular mechanisms are still unclear, this metabolic stress response is probably important in protection from autoimmune pathology. Indeed, preliminary studies suggest that a GCN2 agonist (halofuginone hydrobromide) has potent therapeutic effects in animal models of lupus[89]. Interestingly, GCN2 also mediates the effect of IDO in T cells and inhibits mTORC1 activation[88]. However, a preliminary study in alloreactive human $CD4^+$ T cells has found differences in the immunosupressive activities of rapamycin and a GCN2 agonist, with the latter being more effective at suppressing T_H2 responses[90]. These results suggest that GCN2 activity should be examined in T cells in SLE.

In DCs, mTOR has a central role in integrating activation from TLR signals and growth factors with intracellular nutrient levels[91]. Constitutive mTORC1 activation impaired DC survival and proliferation but accelerated their maturation through Myc proto-oncogene protein (Myc)-dependent metabolic reprogramming that included high levels of ROS production[92]. Given that T cells in SLE have activated mTORC1 and produce high levels of ROS, as well as having chronic endosomal TLR signalling, DCs in SLE probably also have impaired metabolism that enhances their maturation, which in turn contributes to T cell hyperactivation. A study published in 2016 showed that type I interferon production by Toll-like receptor 9 (TLR9)-activated plasmacytoid DCs (pDCs) increased oxidative phosphorylation in an autocrine fashion as well as in neighbouring non-haematopoeitic cells, with a specific contribution of fatty acid oxidation[93], which was necessary for full pDC activation. Interestingly, fatty acids were the major substrate for oxidative phosphorylation in pDCs, and glucose flux and mitochondrial pyruvate uptake to the tricarboxylic acid (TCA) cycle were required to generate citrate for *de novo* fatty acid synthesis. It is now well established that type I interferons are central to SLE pathogenesis. This new

Fatty acid oxidation
A metabolic process that produces ATP from the oxidation of acetyl-CoA derived from the mobilization of fatty acids.

Tricarboxylic acid (TCA) cycle
(Also known as the Krebs cycle) A set of connected pathways in the mitochondrial matrix, which metabolize acetyl-CoA derived from glycolysis or fatty acid oxidation, producing NADH and $FADH_2$ for the electron transport chain and precursors for amino acid and fatty acid synthesis.

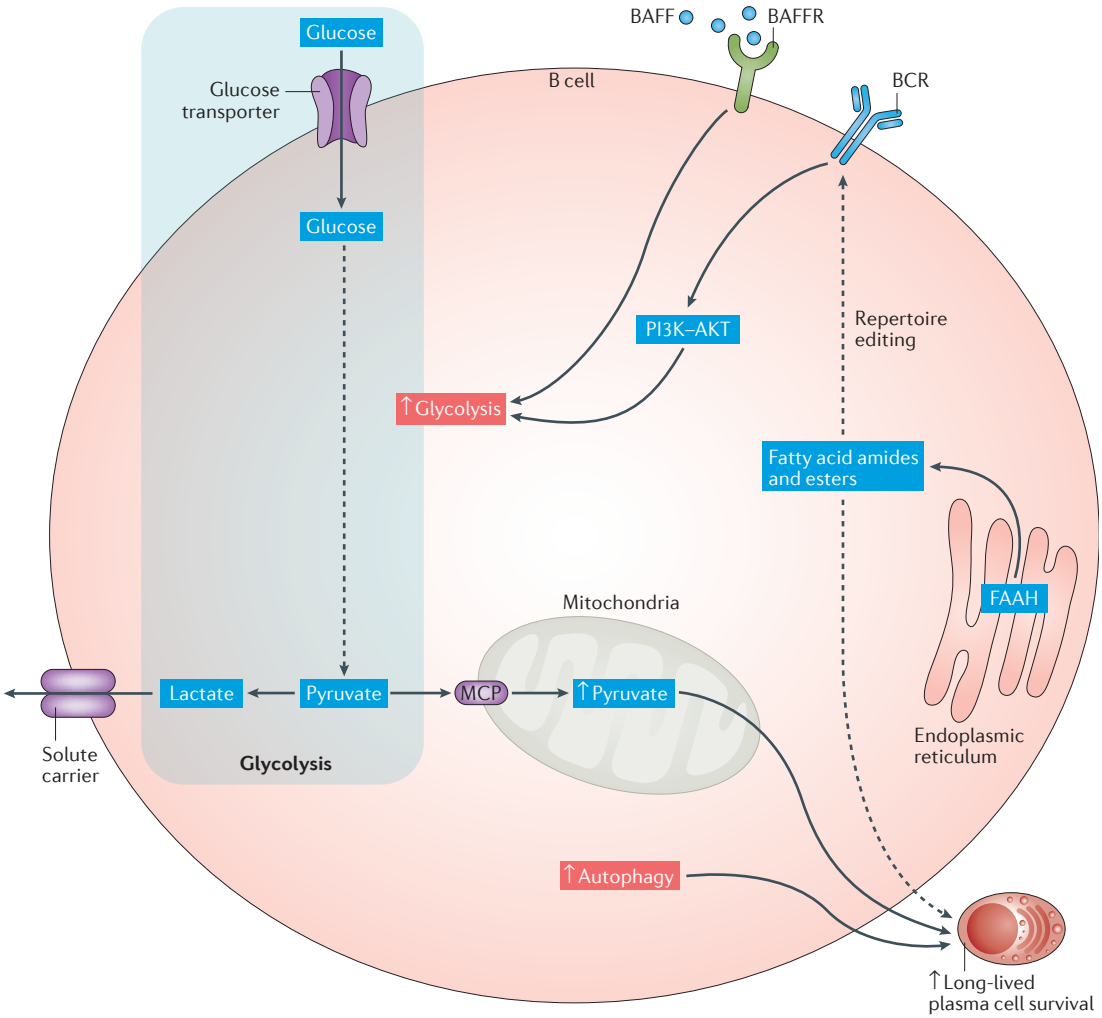

Figure 2 | **Potential metabolic pathways involved in B cells in SLE.** The high level of B cell activating factor (BAFF, also known as BLyS or TNF ligand superfamily member 13B) found in systemic lupus erythematosus (SLE) as well as strong B cell receptor (BCR) signals increase glucose metabolism and glycolysis. This increase might contribute to high levels of pyruvate import into the mitochondria, which are necessary for the survival of long-lived plasma cells. Finally, increased expression of fatty acid amide hydrolase (FAAH) might skew the BCR repertoire towards autoreactive specificities and support plasma cell function or survival. BAFFR, B-cell activating factor receptor; MCP, mitochondrial pyruvate carrier; PI3K, phosphatidylinositol 3-kinase.

metabolic study suggests that inhibition of fatty acid synthesis would decrease type I interferon production by pDCs and therefore have therapeutic benefits. Fatty acid synthesis is also required for T_H17 polarization[94], which suggests that inhibition of fatty acid synthesis could have multiple therapeutic benefits in SLE.

Neutrophils. Neutrophils contribute to SLE pathogenesis through multiple mechanisms, including the production of neutrophil extracellular traps (NETs), which are potent stimulators of type I interferon production[95]. In patients with SLE, ribonucleoprotein immune complexes are potent inducers of NETosis. These immune complexes induce mitochondrial membrane hyperpolarization and the generation of ROS, leading to oxidation of mitochondrial DNA (mtDNA), which is the major immunogenic factor in NETs[96]. Exposure to ribonucleoproteins blocks TFAM (transcription factor A, mitochondrial) phosphorylation, which is a necessary step in the degradation of oxidized mtDNA packed into nucleoids[97]. Consequently, oxidized nucleoids accumulate in the mitochondria of neutrophils in SLE, and are highly interferogenic when extruded in NETs. High levels of mitochondrial ROS also lead to spontaneous NETosis in the low-density neutrophils (LDNs) that are characteristic of SLE. Accordingly, treatment with a mitochondrial-ROS scavenger decreased spontaneous NETosis in MRL/lpr mice and reduced disease activity[96]. Consequently, mitochondrial oxidation is pathogenic in SLE not only through $CD4^+$ T cells, but also through neutrophils. On the other hand, *Nox2*-deficient MRL/lpr mice, which cannot produce NADPH, and consequently have neutrophils that cannot undergo NETosis, develop accelerated disease[98]. This finding is consistent with an increased predisposition to

NETosis
A specialized form of cell death characterized by the release of neutrophil extracellular traps (NETs), which are chromatin structures loaded with granular and nucleic proteins.

autoimmunity, including SLE, in patients with chronic granulomatous disease (CGD), which involves defects in NADPH oxidase. These observations suggest that NETosis could have a protective role in clearing apoptotic debris. However, a 2016 study found that mtDNA oxidation occurs through NETosis of LDNs in both patients with SLE and those with CGD[96]. These conflicting results suggest that mitochondrial oxidation of neutrophils might promote or amplify disease, whereas apoptotic debris clearance by NETosis might prevent disease development. A 2015 study has also shown that asymmetrical ATP production and mTOR signalling are required for neutrophil chemotaxis[99]. Whether the defective ATP production coupled with mTORC1 activation seen in T cells in SLE extends to neutrophils and contributes to their defective functions in SLE is unknown.

Metabolic control of epigenetic regulation

Epigenetic modifications to DNA and histones are impaired at multiple levels in SLE as a consequence, at least in part, of impaired cellular metabolism[100]. DNA hypomethylation in patients with SLE 'de-represses' the transcription of key genes in T cells and increases DNA immunogenicity[101]. The impaired activity of DNA methyltransferases (DNMTs) in SLE can be attributed, at least in part and both directly and indirectly, to oxidative stress[100]. Reduced levels of metabolites derived from methyl group donors have been detected in the sera of patients with SLE[102], which suggests that additional defects in the S-adenosyl-L-methionine (SAM) cycle might contribute to DNA hypomethylation. Epigenetic proinflammatory changes in naive $CD4^+$ T cells from patients with SLE correlated with an enrichment of binding sites for the transcription factor histone-lysine N-methyltransferase EZH2 at methylation sites[103]. EZH2 expression is controlled by the microRNAs miR-26a and miR-101, both of which negatively correlated with SLE disease activity[103]. Interestingly, the expression of these two microRNAs is glucose-sensitive, with increased glycolysis correlating with high expression of EZH2 in a cancer model[104]. This finding suggests that high glucose utilization by T cells in SLE could contribute to their DNA hypomethylation through EZH2 activity, a hypothesis that has yet to be formally verified. These results also suggest that the therapeutic effects of treatment with 2-deoxy-D-glucose, which normalizes some of the phenotypes of T cells in SLE[36], might occur through the epigenome.

Acetyl-CoA is essential for histone acetylation. Overexpression of histone acetyl transferase p300 is protective in lupus-prone mice, but whether acetylation substrate availability (that is, low acetyl-CoA) is involved in SLE is unclear[100]. Histone deacetylases (HDACs) are overexpressed in immune cells, particularly in T cells, from MRL/lpr mice[105], which could be secondary to protein nitration and oxidative stress as histone deacetylation is NAD^+-dependent. Similarly, sirtuins (which are also involved in histone deacetylation) are inhibited by NADPH, which also suggests that HDAC activity would be increased in T cells in SLE. Accordingly, HDAC inhibitors decreased disease severity in lupus-prone mice[106]. Collectively, ample evidence suggests control of SLE at the epigenetic level[107], but connections to the multiple impaired metabolic checkpoints have not yet been clearly delineated.

Role of hypoxia in lupus

Hypoxia and immunometabolism are linked in multiple ways in which the transcription factor HIF-1α dominates[108]. The cellular level of HIF-1α is regulated post-transcriptionally by oxygen-dependent prolyl hydroxylases that tag HIF-1α for ubiquitylation and proteosomal degradation. Under hypoxic conditions, prolyl hydroxylases are inactivated, enabling HIF-1α accumulation. Stabilization of HIF-1α also occurs in pseudohypoxic conditions, such as mTORC1 activation or high levels of mitochondrial ROS[108]. HIF-1α upregulates a large number of key glycolytic genes, such as *GLUT1*, and, accordingly, has a critical role in metabolic reprogramming of immune cells[52]. HIF-1α expression is required for T_H17 and, to a lesser extent, T_H1 cell differentiation[109]. The role of HIF-1α in T_{reg} cell differentiation and function is more complex, and both positive and negative regulatory effects have been reported[52]. In DCs, HIF-1α promotes proinflammatory responses[110]. Consequently, it is likely that HIF-1α drives some of the metabolic abnormalities that have been observed in SLE. Increased *Hif1a* expression has been reported in the $CD4^+$ T cells of lupus-prone mice[35] but, to date, no other study directly linking HIF-1α to lupus has been published. Germinal centre B cells, which are expanded in SLE, are regulated by hypoxia and mTORC1 activity[111]. These effects are complex, however, with variable developmental and regional requirements for HIF-1α and mTORC1 activation in germinal centre B cells. No evidence of an association between SLE and genetic variations in *HIF1A* has been found[112], suggesting that if HIF-1α activity is elevated in SLE, it is a secondary effect, most likely attributable to mTORC1 activation or high levels of mitochondrial ROS.

Reduced angiogenesis is one of the many factors that contributes to hypoxia in kidneys affected by lupus nephritis[113]. A gene expression signature reflecting mitochondrial stress and hypoxia has been reported in the kidneys of BWF1 and NZM2410 mice with chronic disease as well as early in post-treatment relapse; this signature correlated with the level of hypoxia in the kidneys[114]. *Hif1α* expression in podocytes has been linked to NO-induced damage in an immune complex-independent form of lupus nephritis in mice[115]. In addition, the glomeruli and tubules from patients with lupus nephritis as well MRL/lpr mice express high levels of HIF-1α in correlation with mesangial cell expansion[116]. Besides gene expression profiling, metabolic correlates of *Hif1α* expression in renal cells have not been yet established in lupus nephritis.

Systemic contributions to immunometabolism

The metabolic dysfunctions that have been reported in immune cells in SLE most likely have cell-intrinsic origins. However, systemic alterations of metabolism that produce a metabolic imbalance could have a secondary effect on immune cell function. The origin of these

alterations can be genetic or environmental, with variations in gut microbiota probably representing a major source of metabolite imbalance.

Metabolite imbalance. The distribution and abundance of metabolites in serum reflect, at least in part, cellular metabolic programmes. Profoundly dampened serum levels of metabolites derived from glycolysis, fatty acid oxidation and amino acid metabolism were found in patients with SLE[102], possibly reflecting an increased cellular demand for these three metabolic pathways in the immune cells of patients with SLE, as shown in lupus-prone mice[35]. The same study also found a strong presence of markers of oxidative damage, which reflects results obtained in studies of $CD4^+$ T cells from patients with SLE[17]. In addition, imbalances in the lipid profiles of patients with SLE were skewed toward a proinfammatory, prothrombotic state, corresponding to the cardiovascular morbidity often associated with SLE[102]. Two studies have reported a metabolome in peripheral blood lymphocytes of American patients with SLE[41] and in sera of Scandinavian patients with SLE[117] that was different from that of healthy controls. Markers of oxidative stress were common to both studies, with marked decreases in cysteine, NADPH and glutathione levels. In one of these studies, these markers of oxidative stress were reversed by treatment with N-acetylcysteine[41]. This result further supports the notion that oxidation is a major contributor to SLE pathogenesis. Interestingly, these patients also presented with a lower level of amino acids in comparison with healthy subjects, and the most discriminatory metabolite was either decreased tryptophan[117] or increased kynurenine[41], a metabolite generated from tryptophan by IDO. Kynurenine was sufficient to activate mTORC1 in $CD4^+$ T cells from healthy individuals[41], suggesting that this abnormal tryptophan metabolism could enhance $CD4^+$ T cell activation. The increased level of kynurenine in patients with SLE could be the indirect consequence of oxidation, owing to impaired degradation of kynurenine by NADPH-dependent kynurenine hydrolase. In support of this mechanism, N-acetylcysteine treatment, which restores NADPH levels, significantly decreased kynurenine levels in peripheral blood lymphocytes[41]. It has also been shown that type I interferons increase the expression of IDO *in vitro*, skewing tryptophan metabolism towards kynurenine production at the expense of serotonin[118]. Accordingly, patients with SLE and high type I interferon activity had high levels of kynurenine and low serotonin levels[118], the latter offering a potential link to the neurological pathology associated with SLE.

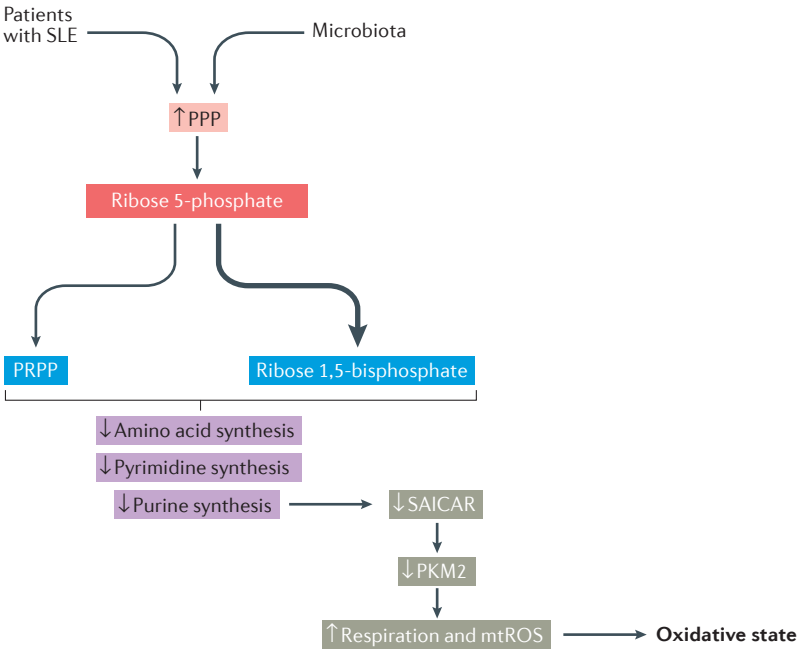

Figure 3 | **Gut microbial metabolites and immune dysregulation in SLE.** Ribose-5-phosphate, produced from an over-reactive pentose phosphate pathway (PPP) in patients with systemic lupus erythematosus (SLE) or from their gut microbiota, is preferentially metabolized into ribose 1,5-bisphosphate instead of phosphoribosyl pyrophosphate (PRPP), which leads to a reduced biosynthesis of amino acids, pyrimidines and purines. Low levels of the purine synthesis intermediate succinylaminoimidazolecarboxamide ribose-5′-phosphate (SAICAR) could result in low glycolytic activity of pyruvate kinase PKM2, which might in turn promote increased production of mitochondrial reactive oxygen species (mtROS), thus contributing to oxidative stress.

Microbiota. Metabolites produced by the gut microbiota contribute to the serum metabolome[119]. Patients with SLE have a distinctive gut microbiota as compared with healthy controls[120,121]. An analysis of foecal metabolites also showed a distinctive 'signature' in patients with SLE as compared with healthy controls, individuals with high BMI or patients with AIDS[122,123]. Which metabolic pathways are responsible for these differences is not well understood. However, a block in the metabolism of ribose 5-phosphate, a product of the PPP (which is a hyperactive pathway in patients with SLE[41]), was uncovered[122]. This block in ribose 5-phosphate processing led to substantially reduced levels of several amino acids, as found in the serum of patients with SLE[117], as well as pyrimidines and purines. Succinylaminoimidazolecarboxamide ribose-5′-phosphate (SAICAR), a purine synthesis intermediate, was absent in faeces from patients with SLE[122]. SAICAR activates the M2 isoform of pyruvate kinase (PKM2), an enzyme that supports glycolysis and HIF-1α transactivation of target genes in tumour cells[124] as well as in immune cells[108]. The status of PKM2 activity, which relies on a balance of cytosolic inactive monomers and metabolically active dimers, as well as nuclear HIF-1α-binding tetramers, has not been explored in SLE or other autoimmune diseases. It is possible, however, that low SAICAR production contributes to the oxidative stress that has been reported in SLE by favouring pyruvate oxidation (FIG. 3).

Overall, serum and faecal metabolites can represent the consequence of dysregulated cellular programmes in immune cells, and might, in turn, have their own direct effects on the functions of these cells, as reviewed above. In addition, it is increasingly clear that many metabolites, such as succinate[125], short chain fatty acids[126] and kynurenine (as shown above), have immunoregulatory properties. Therefore, the metabolite imbalance observed in patients with SLE possibly acts to feed forward the cellular metabolism that sustains immune activation. This process should be further explored with special attention to the contribution of intestinal dysbiosis.

Table 1 | **Metabolic effects of standard-of-care drugs used in patients with SLE**

Drug	Known mechanism	Potential metabolic effect	Refs
Mycophenolic acid (mycophenolate mofetil)	Inhibits guanosine (DNA) synthesis	Reduces AKT–mTOR activation, glycolysis and oxygen consumption in $CD4^+$ T cells	35, 130
Methotrexate	Inhibits purine and pyrimidine (DNA) synthesis	Activates AMPK, which inhibits mTOR activation and glycolysis	133
Glucocorticoids	Immunosupressant	Increase production of leptin, which might activate mTOR	135

AMPK, AMP-activated kinase; mTOR, mechanistic target of rapamycin; SLE, systemic lupus erythematosus.

Metabolic effects of SLE treatments

Many immunosuppressive drugs, including drugs used to treat patients with SLE, have direct or indirect effects on immunometabolism (TABLE 1). Mycophenolate mofetil (MMF), the pro-drug of mycophenolic acid, inhibits inosine monophosphate dehydrogenase type II, a rate-limiting enzyme in the synthesis of guanosine and therefore DNA synthesis. This inhibition leads to immunosuppression by limiting T cell proliferation and promoting apoptosis[127]. Treatment with mycophenolic acid affects a number of key metabolic pathways *in vitro* with the downregulation of *Myc* and *Hif1α* expression in endothelial cells[128] and downregulation of signalling via phosphatidylinositol-3-OH kinase, the serine-threonine kinase AKT and mTOR in gastric tumour cells[129]. More relevant to SLE, mycophenolic acid reduced human $CD4^+$ T cell activation *in vitro* in correlation with a reduction of AKT–mTOR activity[130]. In addition, mycophenolic acid reduced the glycolytic activity of, and oxygen consumption by, human $CD4^+$ T cells activated *in vitro* [35]. mTORC1 activation was found in the glomeruli of BWF1 mice, and rapamycin treatment reduced renal pathology in this lupus model[131]. Therefore, it is possible that treatment with MMF has a beneficial effect on renal pathology through the mTOR pathway. MMF is widely used to treat patients with SLE, but the extent to which its therapeutic effect is linked to immunometabolism, including that of T cells, is currently unknown. Methotrexate, which is discussed in detail in a Perspectives article in this journal[132], increases the intracellular levels of AMP, activates AMPK and inhibits mTOR[133].

Glucocorticoids are known to increase gluconeogenesis, which leads to fat accumulation, and glucocorticoid treatment has been directly linked to increased leptin levels in patients with SLE[134,135]. As leptin can activate mTORC1 (REF. 136), it is possible that glucocorticoid treatments have secondary metabolic effects on immune cells.

In conclusion, experimental evidence suggests that drugs used in the clinic to treat patients with SLE modulate AMPK and mTOR and might therefore have profound effects on immune cell metabolism as part of their therapeutic benefits. Rapamycin, as an mTOR inhibitor, has been used effectively in a proof-of principle study in patients with SLE and is currently being tested in a clinical trial[4,133]. PPARγ agonists also indirectly inhibit mTOR, among many other functions, and the selective PPARγ agonist pioglitazone, which has shown promising effects in preclinical models[84] and in T cells from patients with SLE[137], is currently in clinical trials for SLE with encouraging results[133,138,139].

Conclusions

Studies in patients with SLE and mouse models of the disease have clearly established, using different approaches, that $CD4^+$ T cells have a hypermetabolic state dominated by oxidation, mitochondrial abnormalities and high glucose flux. Furthermore, targeting this T cell metabolism showed therapeutic effects. Reducing mTOR activation with rapamycin[4] or oxidation with N-acetylcysteine[5] reduced disease severity in patients with SLE, and dual inhibition of glucose utilization and complex I activity reversed disease in lupus-prone mice[35,36]. Although most research has focused on $CD4^+$ T cells, other immune cells in SLE are probably affected by metabolic imbalances that might be normalized by these treatments. Deciphering the metabolism of macrophages clearing apoptotic debris and that of germinal centre B cells and long-lived plasma cells might be of particular interest in SLE. There is now evidence that many aspects of metabolic regulation are cell-specific. A better understanding of the many ways in which immune effector functions are regulated by cell-specific metabolic checkpoints could provide more precise and personalized therapeutic options in SLE. In addition to therapeutic potential, the metabolome, potentially intersecting with the microbiota, offers promising venues for disease biomarkers.

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REVIEWS

Immunometabolism in early and late stages of rheumatoid arthritis

Cornelia M. Weyand and Jörg J. Goronzy

Abstract | One of the fundamental traits of immune cells in rheumatoid arthritis (RA) is their ability to proliferate, a property shared with the joint-resident cells that form the synovial pannus. The building of biomass imposes high demands for energy and biosynthetic precursors, implicating metabolic control as a basic disease mechanism. During preclinical RA, when autoreactive T cells expand and immunological tolerance is broken, the main sites of disease are the secondary lymphoid tissues. Naive $CD4^+$ T cells from patients with RA have a distinct metabolic signature, characterized by dampened glycolysis, low ATP levels and enhanced shunting of glucose into the pentose phosphate pathway. Equipped with high levels of NADPH and depleted of intracellular reactive oxygen species, such T cells hyperproliferate and acquire proinflammatory effector functions. During clinical RA, immune cells coexist with stromal cells in the acidic milieu of the inflamed joint. This microenvironment is rich in metabolic intermediates that are released into the extracellular space to shape cell–cell communication and the functional activity of tissue-resident cells. Increasing awareness of how metabolites regulate signalling pathways, guide post-translational modifications and condition the tissue microenvironment will help to connect environmental factors with the pathogenic behaviour of T cells in RA.

Observations made more than 30 years ago introduced the concept of preclinical autoimmunity, which is characterized by the presence of autoantibodies long before the appearance of disease symptoms, thereby fundamentally changing the way that autoimmune disease is understood. This concept, which created a clear separation in time and space between disease onset and clinical manifestations, is now well established in several autoimmune diseases, in particular rheumatoid arthritis (RA)[1,2], systemic lupus erythematosus (SLE)[3] and type 1 diabetes mellitus[4,5]. The idea of preclinical autoimmunity has influenced mechanistic studies and has given rise to the emerging field of preventive immunotherapy to re-induce immune tolerance[6,7].

Immune dysregulation in patients with RA occurs many years before joint inflammation begins[8–10] and is easily detectable in seropositive patients by the presence of antibodies against selected autoantigens. The decisive initial insult is the loss of self-tolerance, a host-protective function guarded by the adaptive immune system. Accordingly, disease-associated genetic polymorphisms identify T cells as key drivers of immune abnormalities in RA[11,12]. Aberrant proliferation, commitment to proinflammatory effector functions, providing help to autoreactive B cells and tissue invasive properties are all phenotypic traits shared by T cells in RA and other chronic inflammatory conditions. These traits impose substantial metabolic demands on T cells, and metabolic reprogramming could have hallmark status in explaining the convergence of phenotypic traits that ultimately result in autoimmune inflammation.

Emerging metabolic patterns in T cells from patients with RA contrast with those in chronically activated healthy T cells, fostering the hope that metabolic programmes delineated in patient-derived cells represent vulnerabilities that can be exploited therapeutically. The inflammatory milieu of the inflamed joint has attracted attention as a site of hypermetabolic activity and high energy needs; however, the molecular features that separate inflammation in rheumatoid joints from other similarly active tissue lesions have not yet emerged. Possible features include molecular signatures for chronically stimulated innate and adaptive immune cells and metabolic profiles derived from stromal components of the joint. Reversing metabolic phenotypes could provide strategies for modulating immune responses with the ultimate aim of reconstituting immune health and intercepting tolerance defects long before joint inflammation occurs.

Major challenges to an integrated view of immunometabolism in RA derive from the fact that the disease process stretches over decades, involves several stages and occurs in multiple tissue environments, including

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Key points

- A fundamental abnormality in rheumatoid arthritis (RA) is the inappropriate growth of immune cells and stromal cells, imposing high metabolic demands to generate energy and biosynthetic precursors
- In RA, immune cells and stromal cells undergo metabolic adaptations to generate biomass
- The disease process in RA involves several stages and multiple tissue sites (such as lymphoid organs and joints), each with a distinct metabolic environment
- A metabolic signature associated with RA involves the dampening of glycolytic flux and the shunting of glucose into the pentose phosphate pathway in $CD4^+$ T cells
- In the rheumatoid joint, metabolic intermediates function as signalling molecules and facilitate cell–cell communication, amplifying inflammatory tissue damage
- The dependence of the rheumatoid disease process on metabolic activity identifies metabolic interference as a potential therapeutic strategy

lymphoid and non-lymphoid organ sites. Although information on immune cell conditioning by different tissue environments is still scant, studies of naive T cell populations not entrapped in the inflamed joints provide insights into primary immune responses and the early stages of RA. The joint lesion in the late stages of RA provides an opportunity to explore how cellular metabolism can condition the tissue milieu and how metabolites can 'moonlight' as intracellular and extracellular signalling molecules. In this Review, we examine emerging data on metabolism in immune cells in seropositive RA and look at how metabolic programmes affect the disease process, focusing on T cells as a key driver of tolerance breakdown.

T cell metabolism in early RA

The early steps of the disease process in RA occur in lymphoid organs, where lymphocytes are primed and differentiate into effector and memory cells. Subsequently, self-reactive T cells and B cells become perpetually activated and expand, releasing cytokines and autoantibodies. In some, but not all, individuals who reach this stage, another protective hurdle is broken; autoreactive T cells and B cells then invade the synovium, functioning as immunopathologic agents by forming organized lymphoid structures and eliciting defective repair mechanisms, supported by myeloid cells, endothelial cells, fibroblasts, chondrocytes and bone cells. Inflammation-induced neoangiogenesis provides easy access for immune cells into the synovial lesion[13,14]. Although it is the most visible battleground, the joint is not the only tissue affected by RA. Throughout all stages of RA, secondary lymphoid tissues supply T cells and B cells to peripheral tissues. Eventually, the spectrum of nonlymphoid organs targeted during RA widens, creating extra-articular manifestations. In all these tissue environments, communication between lymphoid and nonlymphoid cells ultimately determines the activation state, longevity and functional behaviour of the cells involved, and subsequently the tissue damage that is clinically associated with RA. Thus, the pathologic process leading to RA stretches over decades and involves multiple, fundamentally different tissue microenvironments (FIG. 1). Although more information is needed for a contextual analysis of the metabolic environment in lymphoid tissues, progress has been made in understanding intracellular metabolic conditions in naive T cells, thus shedding light on early events in RA pathogenesis.

Over the past two decades, a series of fundamental characteristics have emerged that distinguish T cells from patients with RA and those from age-matched healthy individuals (FIG. 2). Notably, such characteristics extend beyond antigen specificity and include basic biological pathways that enable T cells to transition from protective to auto-aggressive modalities. Central to their role in adaptive immunity, T cells undergo profuse expansion and contraction[15,16]. T cells are able to adapt to severely restrictive bioenergetic conditions[17], as they continue to produce cytokines and proliferate as long as glucose remains available[18]. Creating large amounts of biomass imposes a high demand for energy and biosynthetic precursors[19–21].

Metabolic control of T cell function. Like other proliferative cells, T cells in patients with RA utilize all possible energy sources (sugars, fats and proteins), but glucose remains their major life-sustaining nutrient[22].

Glucose acts as an electron donor. Electron acceptor molecules capture some of the energy released by the stepwise oxidation of glucose and convert it into energy-rich ATP and NADH. One molecule of glucose is broken down into two molecules of pyruvate by the process of glycolysis, with a net gain of two molecules of ATP and two molecules of NADH. The glycolytic pathway is an ancient ATP-producing pathway that is extremely adaptable[23]; under acute energy requirements, glycolytic enzymes can be regulated within minutes. The drawback of this quick regulation is the incomplete oxidation of glucose and the build-up of lactate, which acidifies the cellular and extracellular microenvironments. Under oxygen-rich conditions, pyruvate is transported into the mitochondria and converted into acetyl-CoA to enter the tricarboxylic acid (TCA) cycle[24,25]. This eight-step cycle generates NADH, $FADH_2$ and GTP, which function as electron donors for the electron transport chain. During oxidative phosphorylation, protein complexes in the mitochondrial inner membrane transfer electrons, forming a transmembrane proton gradient and ultimately creating water. T cells, like all cells, harness the energy from this proton gradient to generate ATP, with oxidative phosphorylation yielding 15 times more energy from each molecule of glucose than anaerobic glycolysis[26].

Glycolysis and the pentose phosphate pathway in T cells. Studies using naive $CD4^+CD45RA^+$ T cells from patients with RA have delivered surprising results. Although naive T cells from healthy individuals meet activation-imposed energy demands by upregulating the glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3), induction of this enzyme is reduced in T cells from patients with RA[27,28]. Through its kinase activity, PFKFB3 produces large amounts of fructose-2,6-bisphosphate, which activates phosphofructokinase 1, a rate-limiting glycolytic enzyme. PFKFB3 thus determines the intensity of glycolytic flux and is considered a preferred pharmacologic

Glycolysis
An oxygen-independent metabolic pathway that generates two molecules of pyruvate, ATP and NADH from every one molecule of glucose, supporting the tricarboxylic acid cycle and providing intermediates for the pentose phosphate pathway, glycosylation reactions and the synthesis of biomolecules (including serine, glycine, alanine and acetyl-CoA).

Tricarboxylic acid (TCA) cycle
(Also known as the Krebs cycle) A set of connected pathways in the mitochondrial matrix, which metabolize acetyl-CoA derived from glycolysis or fatty acid oxidation, producing NADH and $FADH_2$ for the electron transport chain and precursors for amino acid and fatty acid synthesis.

Electron transport chain
A series of proteins in the inner mitochondrial membrane that transfer electrons from one to the other in a series of redox reactions, resulting in the movement of protons out of the mitochondrial matrix and in the synthesis of ATP.

Oxidative phosphorylation
A metabolic pathway that produces ATP from the oxidation of acetyl-CoA and the transfer of electrons to the electron transport chain via NADH and $FADH_2$.

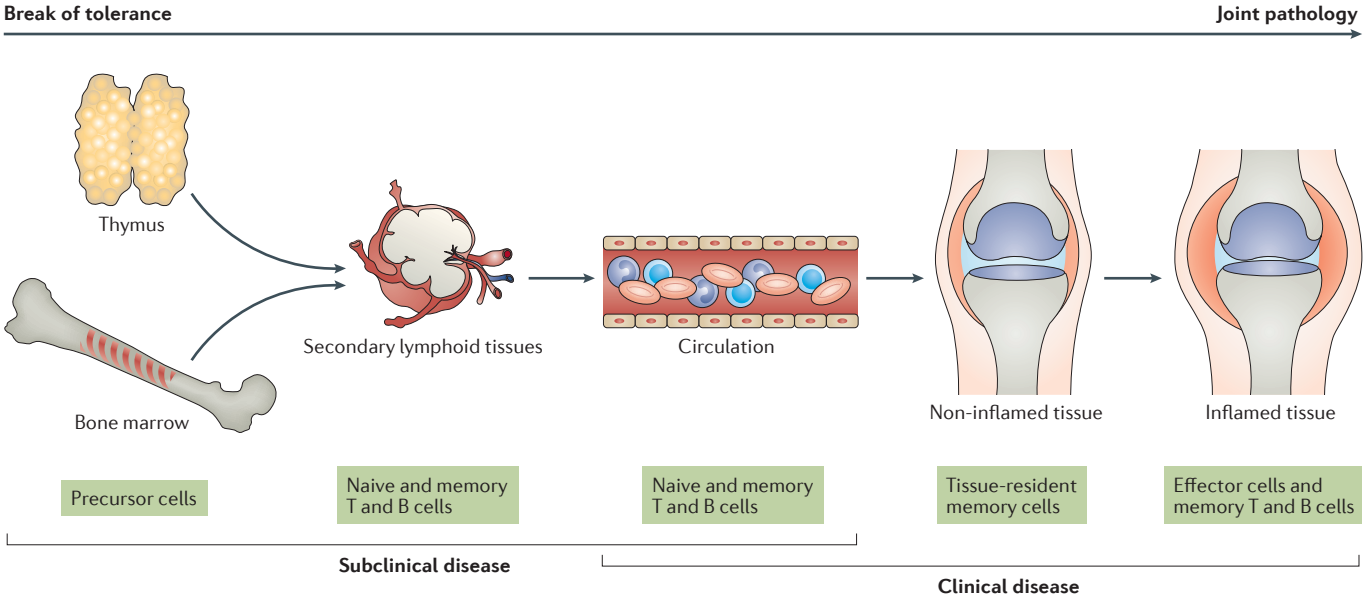

Figure 1 | **Lymphocytes during the disease process of rheumatoid arthritis in different tissue environments.** The disease process of rheumatoid arthritis (RA) involves multiple tissue environments and extends over several decades. An early event is the breakdown of immunologic tolerance, which occurs in lymphoid tissues. After expansion and maturation of autoreactive lymphocytes, autoantigens are encountered in peripheral tissues, eventually leading to the formation of tertiary lymphoid microstructures and chronic destructive inflammation. The inflamed synovial membrane and the disrupted tissue repair response represent the end stage of RA.

target for inhibiting the growth of cancers[29]. PFKFB3 deficiency occurs at an early point in the life cycle of T cells in patients with RA and has profound metabolic and functional consequences (FIG. 3). As a result, lactate production is reduced and ATP levels are lower in such cells than in T cells from healthy individuals[27,28], identifying glycolytic ATP production as a major energy source in $CD4^+$ T cells. In addition to results from patients with RA in the USA[27,28], low ATP production by circulating $CD4^+$ T cells was also seen in patients with RA in a Japanese cohort[30], attesting to the pan-ethnicity of this feature of RA. A low ATP signature might help to identify naive T cells in secondary lymphoid tissues that are prone to autoreactivity. Moreover, T cells from patients with RA have an increased susceptibility to apoptosis and fail to upregulate autophagy as a compensatory pathway for energy generation[27,31,32]. Impaired glycolysis has profound implications for such T cells and their immediate neighbourhood, as low levels of pyruvate production reduces the amount of substrate available for oxidative phosphorylation and thus reduces the generation of reactive oxygen species (ROS). At the same time, low levels of lactate production keep the extracellular pH relatively high and reduced oxygen utilization counteracts hypoxia and its regulatory effects, meaning that these T cells might fail to adapt to tissue hypoxia.

If naive T cells from patients with RA reduce flux through the glycolytic pathway, what is the fate of glucose in these cells? Two side branches of glycolysis, which have a critical role in cellular homeostasis, exist: the hexosamine biosynthesis pathway and the pentose phosphate pathway (PPP). Using glucose, glutamine, acetyl-CoA and uridine, cells can form N-acetylglucosamine for protein O-glycosylation and N-glycosylation through the hexosamine biosynthesis pathway[33,34]. Post-translational modification of proteins by the addition of a single residue of O-linked N-acetylglucosamine has been implicated in regulating the activation of T cells and B cells[35–37]. Whether the post-translational modification of key molecules by the addition of O-linked N-acetylglucosamine differs between T cells from patients with RA and T cells from healthy individuals is currently unknown.

The PPP enables cells to generate products that are crucial for T cell function, as they fuel biomass generation and thus enable T cell expansion. Glucose is converted to glucose-6-phosphate, which enters the PPP to supply the pentose sugars required for nucleotide and nucleic acid synthesis. Glucose-6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme of the PPP and is responsible for the generation of NADPH and ribulose-5-phosphate. NADPH is the cell's most abundant reductive molecule, which, by its electron donor function, holds an important position in regulating the cellular redox status and providing reductive elements for the production of biomolecules. Naive T cells from patients with RA can be distinguished from T cells from healthy individuals by their distinctive shunting of glucose into the PPP (FIG. 3). Compared with T cells from healthy individuals, T cells from patients with RA have increased amounts of G6PD mRNA and protein, and double the level of G6PD enzymatic activity[28]. Consequently, glucose-6-phosphate is shunted into the PPP, generating high amounts of NADPH and reduced glutathione. NADPH is important in controlling lipid synthesis, but the effect of increased PPP utilization in T cells from patients with RA on lipid homeostasis is unknown.

Hexosamine biosynthesis pathway
A side branch of glycolysis used to synthesize nucleotide sugars from fructose-6-phosphate and glutamine, such as uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), which functions as a glycosyl donor for the posttranslational modification of biomolecules.

Pentose phosphate pathway (PPP)
An anabolic metabolic pathway parallel to glycolysis that branches out from glycolysis with the conversion of glucose-6-phosphate to ribose 5-phosphate and generates the reducing equivalent NADPH, ribose-5-phosphate (used in the synthesis of nucleotides and nucleic acids) and erythrose-4-phosphosphate (used in the synthesis of amino acids).

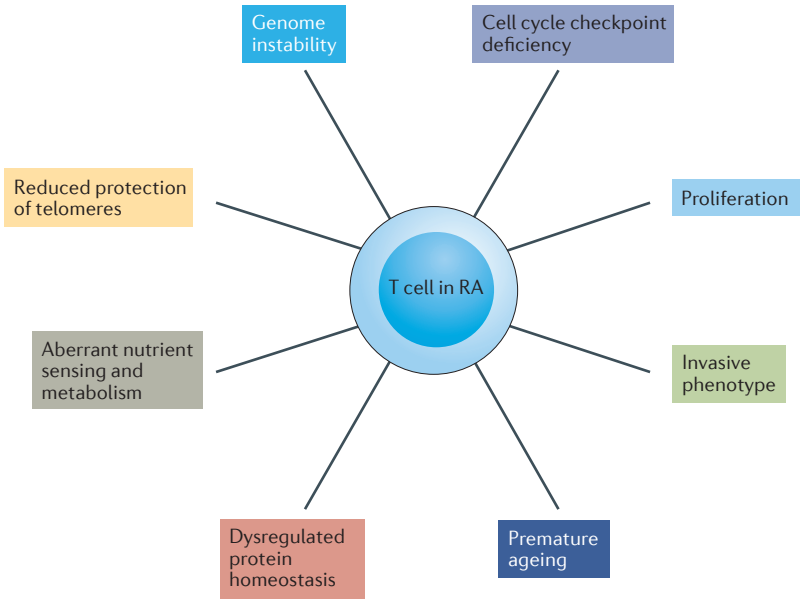

Figure 2 | **Emerging hallmarks of T cells in rheumatoid arthritis.** Hallmarks of T cells in rheumatoid arthritis (RA) are the ability to massively proliferate and to differentiate into proinflammatory effector cells. Several changes in the basic biologic pathways listed here distinguish T cells in patients with RA from those in healthy individuals and enable such T cells to deviate from a protective role to an autoinflammatory one. The molecular defects underlying pathogenic T cell behaviour are currently being discovered; among them is the reprogramming of cellular metabolism, which fuels the functional capabilities of arthritogenic T cells.

Metabolic reprogramming in arthritogenic T cell function. By reducing cellular levels of ROS, PPP shunting affects several aspects of essential T cell functions (BOX 1). Short-lived and highly reactive ROS act as secondary messengers, swiftly altering cellular signalling pathways[38,39]. Basic cellular functions, such as proliferation, differentiation, migration and cell death are now considered to be under the control of ROS[40]. Only if the equilibrium with antioxidant defence systems is disrupted will ROS damage proteins, nucleic acids, lipids, membranes and organelles, eventually triggering cell death. Studies of purified naive T cells from patients with RA and age-matched healthy individuals show that patient-derived T cells are hyperproliferative[27,28], a state inducible in cells from healthy individuals by ROS scavenging[28]. The underlying molecular defect is a shortening of the G2–M phase of the cell cycle. G2–M bypassing occurs when there is insufficient activation of the cell cycle kinase serine-protein kinase ATM, part of a redox-dependent signalling pathway[41]. T cells from patients with RA that have low levels of ATM commit to the type 1 helper T (T_H1) cell and T_H17 cell lineages, rather than differentiating into regulatory T (T_{reg}) cells (BOX 1). In a cohort of patients with RA, the ratio of G6PD to PFKFB3 correlated with clinical disease activity (as measured by 28-joint disease activitiy score), indicating a clinically relevant outcome of diverting glucose into the PPP[28].

Implicating reductive stress, as opposed to oxidative stress, in conferring risk for autoimmune arthritis might be considered contrary to the prevailing dogma, but fits well with groundbreaking work by Holmdahl and colleagues[42,43]. This research group identified neutrophil cytosolic factor 1 (NCF1) and its associated ROS production as major genetic elements in autoimmune arthritis[43]. Previous studies had identified *Ncf1* as a protective gene in rat arthritis[44]. Subsequent work provided detailed mechanistic information on how ROS can suppress inflammatory responses. One of these mechanisms connected macrophage-generated ROS to the suppression of T cell reactivity and to reduced arthritis severity[45]. ROS inhibited pro-arthritogenic T cells by altering thiol groups on the T cell membrane (and possibly in relevant signalling molecules), effectively modulating T cell activation and expansion[46]. ROS deficiency seems to facilitate spontaneous autoimmunity by promoting a type I interferon signature[47]. Notably, sufficient availability of ROS was also required for the induction of T_{reg} cells[48], emphasizing the role of redox signalling in balancing pro-inflammatory and anti-inflammatory immune responses. Thus, redox signalling is critically involved in multiple aspects of T cell biology, with an emerging theme of ROS preventing inappropriate T cell activation[49,50].

Besides activating ATM, ROS serve as secondary messengers in many signalling pathways, including networks that sense the energetic status of a cell and regulate metabolism, such as the mechanistic target of rapamycin (mTOR) and AMP-activated kinase (AMPK) pathways. Coincidentally, mTOR and AMPK also function as master regulators of T cell differentiation and cell fate decisions. As AMPK and mTOR monitor the availability of nutrients, they guide T cells into clonal expansion or reduction and into committed functional lineages and effector functions. By adjusting the activity of AMPK and mTOR, lymphocytes not only match energy supply with demand, but also make decisions about entry into the cell cycle and conversion from naive to memory and terminally differentiated effector cells.

AMPK acts as a redox sensor[51], being activated by increased AMP:ATP ratios, which result in the switching on of catabolic pathways and the switching off of anabolic pathways. Redox conditions predict that patient-derived T cells would not have high levels of activated AMPK (BOX 1). The downstream consequences of a lack of activated AMPK would be profound, as AMPK activation affects several basic cellular functions[52], including glucose uptake, glycolytic flux, mitochondrial biogenesis, fatty acid oxidation, transcriptional activity and cell cycle control. Little is known about the status of AMPK activation in T cells, but ROS deficiency is predicted to paralyze this master integrator of metabolism, proliferation and differentiation. In line with this model, therapeutic AMPK activation reportedly suppresses experimental arthritis[53,54] and methotrexate-mediated activation of an AMPK-dependent pathway is implicated in protecting the vasculature against inflammation[55].

Protein synthesis, cell growth, survival and proliferation, as well as cell fate decisions in differentiating T cells, are under the control of mTOR. Together with AMPK, mTOR is a central communicator, integrating environmental signals with cellular function and differentiation.

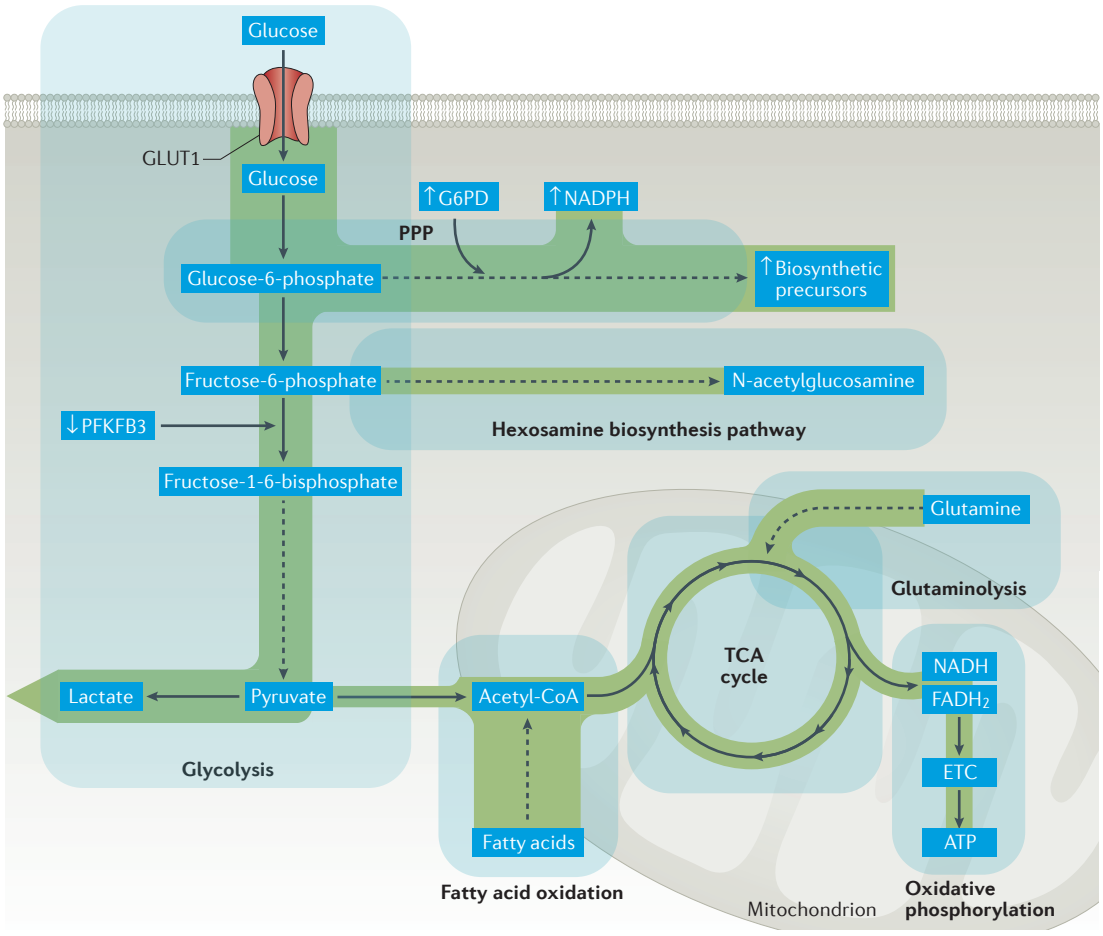

Figure 3 | **Glucose shunting into the pentose phosphate pathway in T cells in rheumatoid arthritis.** Glycolytic breakdown of glucose in T cells in rheumatoid arthritis is reduced as a result of diminished activity of the regulatory enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphophastase 3 (PFKFB3), which curbs ATP production. With increased activity of glucose-6-phosphate dehydrogenase (G6PD), glucose is shunted to the pentose phosphate pathway (PPP), yielding high amounts of the electron donor NADPH and generating biosynthetic precursors. Cellular stores of glutathione are shifted towards the reduced form, cellular levels of reactive oxygen species (ROS) are depleted and cellular oxidant signalling is impaired. ETC, electron transport chain; GLUT1, glucose transporter type 1; TCA, tricarboxylic acid.

Aberrant mTOR activation is associated with cellular senescence, and the mTOR complex 1 inhibitor rapamycin has been investigated as a therapeutic agent to counteract chronic cellular stimulation. mTOR is one of the anchor molecules that interlinks nutrient availability, energy generation and utilization, mitochondrial activity and T cell differentiation. Energy deprivation (as indicated by AMPK activation) and the suppression of mTOR activity are supposed to drive T cells to differentiate into T_{reg} cells[19,56]. However, the precise contribution of different components of the mTOR pathway is not entirely understood, and somewhat contradictory findings regarding the role of mTOR in T cell biology have fuelled discussions within the scientific community[57]. The ability of mTOR to integrate the regulation of nutrient supply, bioenergetics and T cell differentiation makes it a promising target for therapeutic intervention to suppress abnormal T cell differentiation during the early stages of RA[58–61].

A common factor of the crosstalk between extracellular and intracellular cues seems to be ROS, which swiftly adapt cellular functions to the immediate tissue environment and the needs of the host. The reliance of T cells on ROS-dependent signals is likely to be particularly important in microenvironments with low levels of ROS, such as well-oxygenized secondary lymphoid organs. In the arthritic joint, ROS are abundant and participate in the feed-forward amplification of tissue damage[62]. Although ROS are needed to prevent emerging autoimmunity, ROS scavenging might be beneficial in lowering the inflammatory burden in the arthritic synovium. The multiple functions of ROS represent excellent opportunities to target different stages of RA therapeutically.

Immunometabolism in late RA

In the later stages of RA, the disease process migrates from the secondary lymphoid tissues to peripheral tissue environments, in particular the synovial lining of

Box 1 | **Functional consequences of metabolic reprogramming in T cells in RA**

Alterations in how T cells in patients with rheumatoid arthritis (RA) utilize glucose and generate biosynthetic precursors has profound implications for intracellular signalling pathways and, eventually, differentiation into immune effector cells. Definition of the underlying molecular defects in such T cells has revealed novel therapeutic opportunities based on 'rewiring' metabolic networks.

Metabolic reprogramming of T cells in RA

- $CD4^+$ T cells from patients with RA have markedly reduced glycolytic activity[27], resulting in low levels of ATP and lactate
- These T cells shunt glucose to the pentose phosphate pathway[28], generating high levels of NADPH and an abundance of biosynthetic precursors
- Excess NADPH leads to the accumulation of reduced glutathione and the depletion of cellular reactive oxygen species, impairing oxidation-dependent signalling pathways

Effect of metabolic constraints on cellular signalling pathways

- Defective activation of the cell cycle kinase serine-protein kinase ATM[27,28]
- Defective activation of the energy sensor AMP kinase (AMPK)[136]
- Aberrant activation of mechanistic target of rapamycin complex 1 (mTORC1)[136,137]

Functional consequences of intracellular signalling abnormalities

- Bypass of the G2–M cell cycle phase, leading to hyperproliferation[27,28]
- Early naive-to-memory T cell conversion[28]
- Hypermigration of T cells[28,129]
- Enhanced tissue invasion by T cells[28,129]
- Biased T cell differentiation, favouring type 1 T helper (T_H1) cells and T_H17 cells, but restricting regulatory T cell differentiation[28]

Therapeutic opportunities

- Improve T cell redox signalling by using, for example, a γ-glutamylcysteine synthetase inhibitor or a redox-cycling agent
- Restore ATM activation
- Normalize AMPK activation

diarthrodial joints. Here, T cells, B cells and plasma cells, together with specialized antigen-presenting cells, create organized lymphoid structures[63,64] and interact with tissue-resident cells such as fibroblast-like synoviocytes (FLSs), endothelial cells, macrophages, neuronal cells, chondrocytes and bone cells[65]. The synovial pannus has tissue-destructive and invasive properties[66,67], with features of a non-healing tissue wound. Fundamental biologic processes of the synovial pannus such as sustained proliferative signalling, angiogenesis, cellular de-differentiation and unbalanced bone turnover are associated with high metabolic demands in all cell types involved. Overall, the inflamed joint in patients with RA is a hypermetabolic lesion.

Hypermetabolic activity of immune and stromal cells creates a tissue environment that in itself can regulate cellular behaviour. Tissue-infiltrating immune cells, specifically T cells in the joints of patients with RA, should encounter chronic stimulatory conditions, which in turn should lead to exhaustion and cellular senescence[68–71]. This aspect of T cell biology in RA is not fully understood. Of similar importance is the recognition that hypermetabolic cells release metabolic intermediates into their environment that are sensed by neighbouring cells, thus shaping the intensity, duration and type of inflammation present. A better understanding of such metabolic intermediates and their proinflammatory functions could broaden the potential therapeutic targets in established RA, extending beyond the successful blockade of end-stage inflammatory mediators, particularly proinflammatory cytokines such as TNF and IL-6 (REFS 72,73).

Tissue oxygen. By engulfing aerobic prokaryotes, which eventually became mitochondria, eukaryotic cells acquired the ability to utilize oxygen for energy production, and tissue oxygen levels became a major regulator of bioenergetics. First investigated in cancer cells, which have high energy needs, hypoxia-inducible factor 1α (HIF-1α) senses and connects oxygen availability to metabolic activity and ATP production, and holds a key position in both aerobic and anaerobic glycolysis[74,75]. In tumours, HIF-1α is responsible for excessive angiogenesis, clonal selection of tumour cells and the enforcement of metabolic adaptations. Tumours often have anoxic regions and extreme hypoxic gradients throughout the tumour tissue (ranging from 0.1–6% pO_2)[76]. Although there does not seem to be a single hypoxic threshold that can be applied generally, oxygen levels required for hypoxia-induced gene expression are estimated to be in the range of 1–15 mmHg (REF. 77). The inflammatory lesion of the rheumatic joint is considered to be a hypoxic site, in which HIF-1α functions as a metabolic inducer[78,79]. Low tissue oxygen levels are implicated in inducing mitochondrial dysfunction and in promoting a switch to glycolysis[62,80]. Biniecka and colleagues arthroscopically measured tissue oxygen tensions in the synovium of patients with inflammatory arthritis and correlated tissue hypoxia with the induction of glycolytic activity[80]. In a group of six patients, synovial oxygen partial pressures increased from <20 mmHg to >20 mmHg upon response to treatment with TNF inhibitors, whereas a group of 13 patients who did not respond to TNF inhibitor treatment had median tissue oxygen levels of >20 mmHg before and after TNF blockade. These data indicate that the inflamed synovial lining is not as hypoxic as tumour tissues, in line with the clinical observation that in the inflamed synovium, tissue expansion outweighs tissue death. Hypoxia and excessive angiogenesis are both considered to be proinflammatory, raising the question of whether therapeutic targeting should aim to increase or reduce tissue oxygen supply[81].

Glycolytic intermediates. The acidification of the rheumatoid joint (and other RA-associated exudates) has fascinated the scientific community for more than 50 years[82,83], giving rise to the idea that low glucose levels and high lactate levels could have diagnostic value. Work from the early 1970s showed higher mean oxygen uptake rates and higher mean rates of lactate appearance in saline deposited into the joints of patients with RA compared with that in the joints of patients with degenerative joint disease[84]. Studies from the past few years have confirmed the decline in glucose and the appearance of lactate in synovial fluid[85], which are compatible with the

requirement of the tissue lesion to utilize glucose for fast access to energy. FLSs shift their metabolism towards anaerobic glycolysis[86] and are especially efficient in exporting lactate into the extracellular space[87], where it acidifies the microenvironment and also participates in regulating the function of surrounding cells (FIG. 4). Local effects of lactate depend upon its concentration, as well as the ability of tissue-resident cells to sense and take up acids. Acid-sensing ion channels (cation channels activated by extracellular acid) are implicated in acid-induced cell injury, such as during chondrocyte apoptosis[88]. Tissue-resident T cells are also affected by an environment that contains lactate and might contribute actively to this environment by exporting lactate themselves[68]. When exposed to sodium lactate or lactic acid, the motility of $CD4^+$ and $CD8^+$ T cells is inhibited, possibly prolonging the retention of tissue-infiltrating T cells[89]. Additionally, lactate promoted IL-17 production by $CD4^+$ T cells and induced a loss of cytolytic function in $CD8^+$ T cells[89]. Taken together, these results suggest that the switch towards anaerobic glycolysis in synovial cells might sustain proinflammatory amplification loops and contribute directly to cellular injury (FIG. 4).

In contrast to lactate, which seems to have mainly proinflammatory functions, another intermediate of the glycolytic pathway, fructose 1,6-bisphosphate, exhibits strong anti-inflammatory properties. A single treatment with fructose 1,6-bisphosphate markedly suppressed arthritis in two animal models[90]. The protective mechanism has been linked to improved generation of ATP, which is then hydrolysed by the nucleotidases CD39 and CD73 to produce adenosine[90]. These results support the observation that the low level of ATP in T cells from patients with RA is ultimately proinflammatory[27,28].

Mitochondrial intermediates. Increased rates of oxygen uptake in the RA joint[84], helped by the excessive angiogenesis known to occur at this chronically stimulated

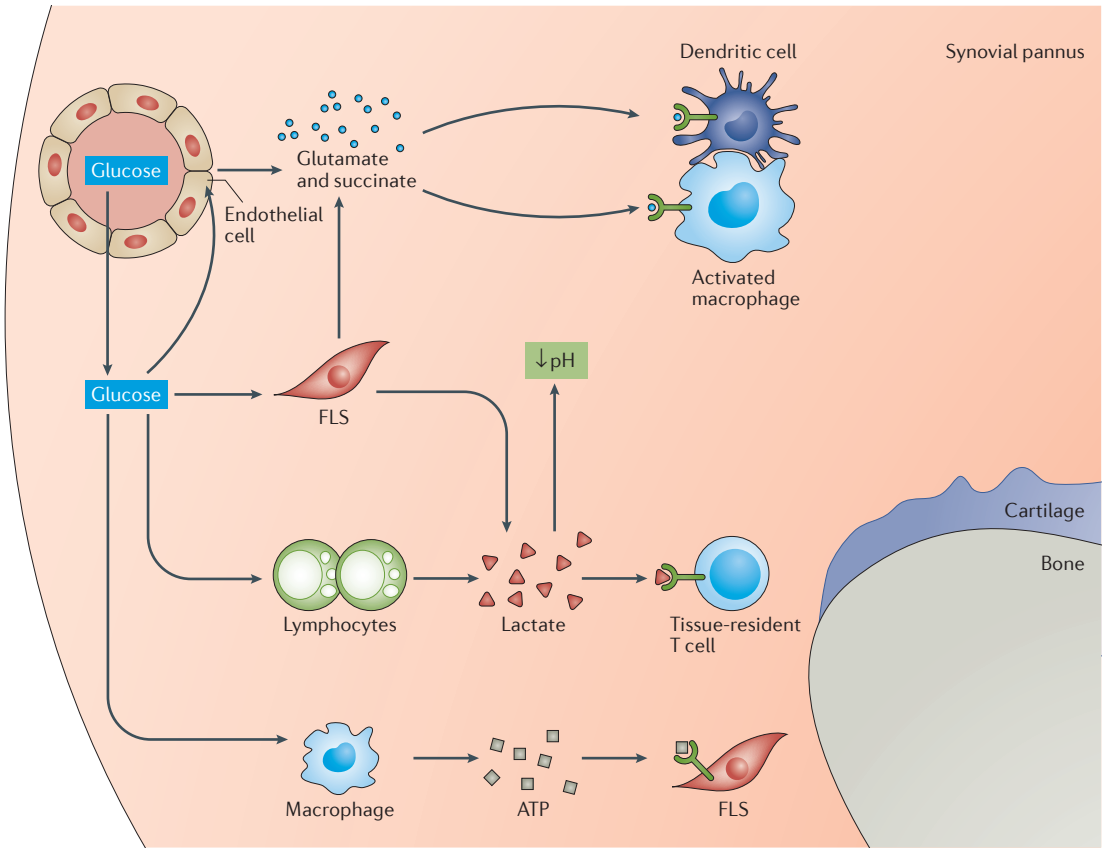

Figure 4 | **Metabolic intermediates in the rheumatoid joint.** The synovial pannus in the joints of patients with rheumatoid arthritis is a hypermetabolic lesion that demands high amounts of nutrients and oxygen to fulfil the energy and biosynthetic needs of its proliferative cells. The presence of glucose enables rapid and adaptive production of ATP, even under hypoxic conditions. Metabolic products such as lactate and ATP are released into the extracellular space, where they promote cell–cell communication and regulatory control. Lactate acidifies the tissue microenvironment and might directly contribute to cellular injury. With high levels of mitochondrial activity in tissue-resident and invasive cells, intermediates of the tricarboxylic acid cycle such as succinate and glutamate are secreted into the extracellular space. Signals transduced through specialized receptors (such as hydroxycarboxylic acid receptor 1 (also known as GPR81) for lactate and succinate receptor 1 (also known as GPR91) for succinate) regulate the functions of cells that sense extracellular metabolites. In this situation, metabolites serve as signalling molecules in cell–cell communication and in microenvironmental surveillance. FLS, fibroblast-like synoviocyte.

tissue site[91,92], indicate that mitochondrial metabolism remains intact and contributes to ATP generation. Mitochondria not only consume oxygen to generate ATP, but also produce metabolic intermediates of the TCA cycle that participate in a multitude of metabolic pathways[93]. The concentrations of several of these TCA cycle intermediates, including succinate, citrate, glutamate, fumarate and aspartate, are altered in continuously proliferating cancer cells[94]. The same mitochondrial intermediates are also enriched in synovial fluid[95] and, like lactate, have functions beyond their role as anabolic metabolites[96–98]. The presence of such TCA cycle intermediates in synovial fluid suggests that the synovial pannus represents an oxygenated environment with ample access to sources of carbohydrates, amino acids and lipids.

Several TCA cycle intermediates have been identified as potential amplifiers of inflammatory activity and as possible therapeutic targets[99–102] (FIG. 4). Glutamate concentrations are elevated in arthritic joints; the triggering of glutamate receptors increases the release of IL-6 and induces arthritic pain[103]. Synovial fluid from patients with RA also contains an abundance of succinate, a product released into the extracellular milieu by activated macrophages. Interestingly, such macrophages express succinate receptor 1 (also known as GPR91), through which they sense succinate levels and induce inflammasome activation[104]. Previously, succinate has been demonstrated to modulate dendritic cell function by triggering succinate receptor 1 (REF. 105) and to induce IL-1β production in lipopolysaccharide-treated macrophages by stabilizing HIF-1α[106]. Another important product of the mitochondria of activated tissue macrophages is ROS, which remodel inflammatory signalling networks and sustain IL-1β and IL-6 production by enhancing the phosphorylation of signal transducer and activator of transcription 3 (REF. 107).

Alarmins. Cells under stress actively or passively release endogenous danger signals (known as alarmins), including high mobility group protein B1 (HMGB1), S100A proteins, heat shock proteins and purine metabolites (such as uric acid and ATP). Innate and adaptive immune cells sense such extracellular alarmins through specific receptors, which trigger and fine-tune inflammatory and repair responses[108,109]. ATP, produced by glycolysis or oxidative phosphorylation in the mitochondria, is exported into the extracellular space, where it has been associated with anti-inflammatory activity (FIG. 4). Lymphocytes from patients with RA have high levels of CD39 activity, which could lead to insufficient preservation of extracellular ATP[110]. Conversely, ATP-dependent activation of the P2X7 purine receptor on mast cells upregulates protein arginine deiminase[111], an enzyme involved in the conversion of arginine residues into citrulline, a post-translational modification considered to be arthritogenic in RA. P2X7 is found on human FLSs[112], enabling these cells to closely monitor ATP levels in the extracellular milieu. In general, by monitoring ATP levels cells can closely assess the metabolic activity and the metabolic pathways preferred by their neighbouring cells. Expression of CD39 on T cells seems to be particularly important for memory cells and is a marker of T cell ageing[113].

DNA, of both nuclear and mitochondrial origin, can acquire alarmin function when released into the extracellular space, where nucleic acids can engage pattern-recognition receptors on surrounding cells[114]. This mechanism could enable the communication of metabolic stress, as synovial cells sense not only mitochondrial intermediates but also mitochondrial DNA[115].

Other inflammatory networks. A number of inflammatory processes that are ultimately dependent on energy supply and the metabolic environment contribute to the pathogenesis of RA, as permissive factors and as aggravators of tissue injury. Abnormalities in autophagy are suspected to determine cellular hyperplasia as well as cell loss[116]. PFKFB3, the glycolytic enzyme implicated in metabolic reprogramming of T cells in the early stages of RA[31], is also linked to the control of autophagy[32]. The identification of genetic mutations in inflammasome activation pathways in patients with autoinflammatory syndromes has also been instructive in connecting molecular networks to inflammatory outcomes, and has highlighted the multiple roles of mitochondria in regulating inflammation[117,118].

Metabolomics in RA

Progress in technologies such as mass spectrometry and chromatography that enable increased precision of mass detection and metabolite identification have driven interest in the measurement of hundreds of metabolites in cells, tissues and fluids. In patients with RA, plasma, serum, synovial fluid and synovial tissue have all been utilized for metabolomics studies[119–122]. No unifying metabolic marker(s) has been discovered, but some common signatures have emerged. Using metabolomics, patients can be differentiated from healthy controls, anti-inflammatory treatments can be seen to affect metabolite patterns, and patients in therapy-induced remission can still be distinguished from healthy individuals[119–122] (which shows that current therapies improve but do not cure underlying pathologies). Importantly, these studies show that inflammation in patients with RA is a complex process, extending far beyond lactate production. Although less efficient than oxidative phosphorylation, the evolutionarily ancient process of anaerobic glycolysis can be upregulated within minutes. Metabolomics studies in patients with RA have revealed that a switch to aerobic glycolysis, the so-called Warburg effect, is not enough to capture all metabolic adaptations associated with the disease process. In a 2010 study by Lauridsen *et al.*[123], increased amounts of cholesterol and unsaturated lipids and a decreased amount of HDL cholesterol distinguished patients with RA from healthy individuals. Decreased lipid signals were also the major discriminators between patients with RA and healthy individuals in a 2013 study by Young *et al.*[124]. These authors also identified high levels of 3-hydroxybutyrate as a marker for RA, suggesting increased lipolytic activity[124].

Warburg effect
The high utilization of glycolysis by rapidly proliferating cells and the subsequent release of lactate into the extracellular milieu; a phenomenon first desribed by Otto Warburg.

A 2016 study highlighted increased levels of fatty acids and cholesterol and decreased levels of amino acids and glucose in patients with RA[125]. Another signature reported for metabolites in the blood of patients with RA includes low levels of valine, isoleucine, alanine, creatinine, histidine and lactate and high levels of 3-hydroxyisobutyrate, acetate, N-acetylcysteine, acetoacetate and acetone[126]. Fatigue in patients with RA is associated with downregulation of metabolites from the urea cycle (such as fatty acids, tocopherols, aromatic amino acids and hypoxanthine)[127].

Collectively, metabolomics studies provide many opportunities both clinically and conceptually, yet the data from these studies have not yet been used to their full effect. Part of the problem lies in the heterogeneity of cells and tissues that actively or passively release metabolites that accumulate in body fluids. The functional effects of such metabolites are dictated by their abundance in tissue microenvironments, the activity of transport mechanisms and the diversity of cell types that generate and use them. Increased levels of a metabolite might indicate a lack of utilization or compensatory production, whereas decreased levels might result from disproportionate utilization or insufficient generation. To define evidence-based pathways for potential clinical translation, researchers will need to use functional metabolomics studies to evaluate how defined metabolic products affect cellular and multi-cellular homeostasis.

An obvious question is whether the analysis of metabolites in the inflammatory environment, as captured in synovial fluid or tissue, yields novel insights into the nature of the inflammation. An abundance of succinate, aspartate, glutamate and citrulline in synovial fluid from patients with RA attests to the intense levels of mitochondrial metabolic activity[95]. Conversely, levels of isopalmitic acid, glycerol, myristic acid, palmitoleic acid, hydroxylamine and ethanolamine are low in patients with RA[100,104].

Conclusions

As a prototypic autoimmune disease, RA begins with the immune system making a fundamental mistake — not being able to distinguish self from non-self — with the end result of relentless inflammation in the synovial microenvironment. Although the location, intensity and specific pathways of disease vary over the lifetime of the patient, tolerance breakdown, subclinical RA and clinical RA share the need for cellular expansion and biomass generation. How cells fulfil the consequent demands for cellular energy and biosynthetic precursors has emerged as a critical domain in autoimmune inflammation.

The early stages of RA, in which T cells break the state of tolerance and provide help to autoantibody-producing B cells, unfold in lymphoid tissues and involve cells that have not yet made a lineage commitment. Metabolically, naive T cells from patients with RA are dysregulated and respond to activation by shunting glucose into the PPP. Such T cells generate low levels of ATP and lactate, but have excess NADPH and biosynthetic precursors. Consequently, these T cells have reduced levels of intracellular ROS, impaired redox signalling and insufficiently activate the cell cycle kinase ATM. The result is a hyperproliferative T cell that is biased towards T_H1 and T_H17 effector cell functions. Although the underlying defects that lead to metabolic reprogramming are not fully understood, divergence of glucose utilization towards synthetic and proliferative functions is now recognized as part of the DNA repair and T cell ageing programme[128]. Premature ageing in T cells from patients with RA is connected to telomere instability. Reduced activity of the DNA repair nuclease MRE11A induces a cellular senescence module that is defined by gain of the cell cycle regulators p16 and p21 and the cell surface receptor CD57 (REF 129). Shunting glucose towards the PPP and telomeric uncapping are both directly implicated in the tissue invasive and proinflammatory properties of T cells from patients with RA, indicating shared upstream abnormalities[27,129].

The metabolic environment of the inflamed joint is demonstrably altered, in line with the high metabolic activity of chronically active lymphoid and stromal cells. Proliferating stromal cells use glucose as an energy source and acidify the tissue microenvironment by releasing lactate. Excess TCA cycle intermediates suggest high levels of mitochondrial activity, possibly fuelled by angiogenesis delivering oxygen to the tissue. Extracellular glutamate and succinate have been linked to proinflammatory functions, exemplifying the increasingly recognized role of such metabolites as signalling molecules, above and beyond their anabolic contributions[130,131].

An obvious motivation to better understand the metabolism of inflammatory immune cells is the idea that tailored exercise programmes, dietary habits and selected nutrients might be applied in the management of RA. Omega-3 fatty acids, moderate alcohol consumption and strict adherence to a Mediterranean diet reportedly have beneficial effects on RA disease activity[132], supporting the notion that dietary interventions could be developed as additional immunomodulatory treatments for patients with RA. Molecular studies and well-designed clinical trials will help with the design of disease-specific recommendations. Conversely, clarifying how immunosuppressive drugs that successfully treat RA, such as methotrexate, chloroquine and TNF inhibitors, interfere with metabolic processes could provide useful clues as to how metabolism is connected to RA and its comorbidities[133]. Evolving therapeutic strategies that exploit metabolic regulation as a therapeutic target in rheumatic diseases are reviewed in another article in this journal[134].

Analysis of the metabolic pathways in RA widens the pathogenic concept of this disease beyond a narrow view of autoimmunity triggered by the recognition of an autoantigen. An increased knowledge of metabolism promises to yield insights into the relationship between genes and environment in the decades-long disease process, to enable an integrated view of host–microbiota interactions and to invigorate the discussion of nutraceuticals as novel therapeutic agents[135].

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REVIEWS

The role of metabolism in the pathogenesis of osteoarthritis

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Abstract | Metabolism is important for cartilage and synovial joint function. Under adverse microenvironmental conditions, mammalian cells undergo a switch in cell metabolism from a resting regulatory state to a highly metabolically activate state to maintain energy homeostasis. This phenomenon also leads to an increase in metabolic intermediates for the biosynthesis of inflammatory and degradative proteins, which in turn activate key transcription factors and inflammatory signalling pathways involved in catabolic processes, and the persistent perpetuation of drivers of pathogenesis. In the past few years, several studies have demonstrated that metabolism has a key role in inflammatory joint diseases. In particular, metabolism is drastically altered in osteoarthritis (OA) and aberrant immunometabolism may be a key feature of many phenotypes of OA. This Review focuses on aberrant metabolism in the pathogenesis of OA, summarizing the current state of knowledge on the role of impaired metabolism in the cells of the osteoarthritic joint. We also highlight areas for future research, such as the potential to target metabolic pathways and mediators therapeutically.

Immunometabolism is an emerging field at the interface of immunology and metabolism, which, historically, have been considered distinct disciplines[1,2]. Focusing on changes in the intracellular metabolic pathways of immune cells, and how these alterations modulate cellular function[3], immunometabolism highlights the key role of metabolic reprogramming within the immune system in the pathogenesis and progression of chronic inflammatory diseases. Evidence suggests that six major metabolic pathways are involved in immunometabolism, including glycolysis, the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway (PPP), fatty acid oxidation, fatty acid synthesis and amino acid metabolism[3]. Changes in the levels of metabolites in these pathways act as important metabolic switches with the capacity to shape the ways in which immune cells respond to their environment.

Interest in this new field of research is steadily gaining momentum owing to the discovery that underlying metabolic disturbances in obesity-induced inflammation, insulin resistance and type 2 diabetes mellitus have the potential to promote a variety of chronic diseases and comorbidities[1,4]. Inflammatory diseases, including osteoarthritis (OA) (which is now appreciated to involve low-grade inflammation), are associated with a sedentary lifestyle, physical inactivity, obesity and inflammaging; in OA, poor diet, obesity and physical inactivity directly contribute to metabolic changes that promote inflammaging and cellular senescence[5,6] (FIG. 1). According to the immunometabolic hypothesis, aberrant metabolism, inflammatory mediators and disturbed circadian rhythms and biological clocks are intimately involved in many inflammatory responses[7]. The ability to control and manipulate cellular metabolism could, therefore, lead to new approaches for treating inflammatory diseases.

Evidence is emerging for a key role for metabolism in the regulation of inflammatory responses and immune cell function, with different immune cells showing distinct metabolic signatures that regulate their biological responses[8]. However, the same principle can also be applied to many non-immune cell types. Under adverse conditions, most mammalian cells undergo a shift in energy metabolism from a resting regulatory state to a highly metabolically active state to maintain energy homeostasis and promote cell survival[9]. This metabolic shift normally occurs when oxygen levels are low, limiting the metabolism of pyruvate by the tricarboxylic acid cycle in mitochondria during oxidative phosphorylation. However, in some instances this shift can occur under aerobic conditions (known as the Warburg effect), as in cancer and many degenerative and inflammatory conditions, and represents a potential threat to cell function and survival. Such a metabolic shift is now believed to occur in the articular cartilage, subchondral bone and synovium of the joints of patients with OA, influencing

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Key points

- Metabolism has a key role in the physiological turnover of synovial joint tissues, including articular cartilage
- In osteoarthritis (OA), chondrocytes and cells in joint tissues other than cartilage undergo metabolic alterations and shift from a resting regulatory state to a highly metabolically active state
- Inflammatory mediators, metabolic intermediates and immune cells influence cellular responses in the pathophysiology of OA
- Key metabolic pathways and mediators might be targets of future therapies for OA

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the metabolic behaviour of chondrocytes, synoviocytes and bone cells, as well as their interactions with the immune system through synovial macrophages[10]. There is also increasing and overwhelming evidence to suggest that OA is a metabolic disorder[11–13]. In this Review, we focus on aberrant metabolism in OA, summarizing the current state of knowledge in this area by focusing on metabolic aspects of synovial joint tissue and cell function, and examining the evidence for deregulated metabolism in chondrocytes and synoviocytes in OA. We also propose priority areas for future research, in particular metabolic pathways in synovial fibroblasts and activated macrophages, about which little is currently known.

OA as a metabolic disorder

OA, an age-related low-grade inflammatory disease of the synovial joints[14,15], is one of the most costly and disabling forms of arthritis, being more prevalent than rheumatoid arthritis (RA) or other arthritic diseases and representing a major public health burden[16]. OA is characterized by the progressive deterioration of articular cartilage and structural changes to the entire synovial joint, including the synovium[17], meniscus (in the knee)[18], periarticular ligaments[19], adipose tissue (for example, the infrapatellar fat pad in the knee)[20] and subchondral bone[21]. These deleterious structural changes in articular tissues impair the functional integrity of the synovial joint[22], adversely affecting its biomechanics and attenuating its already limited inherent capacity for repair and regeneration[23]. Although OA was historically viewed as a 'wear and tear' disease, it is now generally accepted to be a low-grade inflammatory disease[24,25] affecting the whole joint[11,15,26,27]. The pathogenesis and progression of OA seem to be the result of the complex and dynamic interplay of mechanical, cellular and systemic molecular factors[26]. Many of the biochemical mediators involved in OA have important systemic and immunoregulatory roles[28], including several complement proteins that are implicated in low-grade inflammation[25], providing new evidence for key molecular and metabolic factors as drivers of OA.

Notably, OA is not a homogeneous disease but is in fact highly heterogeneous, characterized by a number of different phenotypes (including a distinct metabolic phenotype), each of which is thought to have different drivers (FIG. 2). The various phenotypes of OA have important differences, but are likely to share key elements such as ageing, biomechanical factors and metabolic alterations. Although this idea complicates traditional approaches for developing new treatments, it also presents opportunities for developing therapies targeted to each phenotype.

Preclinical research in animal models of OA and clinical studies in patients with OA have shown that age[14], obesity[29] and metabolic syndrome[30] are major risk factors for the development of OA[11,31]. Obesity is the strongest risk factor for disease onset in the knee[32], and mechanical factors (such as ambulatory load) dominate the risk for disease progression[26,33]. However, the fact that obese individuals have an increased risk of developing OA in non-weight-bearing joints such as the hands and wrists[34] suggests that factors produced by white adipose tissue (WAT) might have a role in the onset and/or progression of OA. Increased adiposity and dysfunction of WAT is closely related to the chronic low-grade inflammatory status that is a systemic feature of both obesity and OA[35–37]. A consequence of this dysfunction is that WAT adopts an atherogenic, diabetogenic and inflammatory profile, producing proinflammatory factors (known as adipokines) that promote inflammation and the degradation of cartilage and thus affect the whole joint microenvironment[38], including the activity of immune cells in patients with OA[39]. Accumulating evidence also points to the infrapatellar fat pad as another potential source of proinflammatory adipokines in the joint[20]. Chondrocytes, synoviocytes, adipocytes, macrophages and other types of cell in the fat pad are likely to collectively contribute to the production of proinflammatory cytokines and chemokines. Therefore, tackling obesity[29] and the underlying causes of metabolic syndrome[30] through lifestyle changes (such as improved diet, increased physical activity and weight loss) has been proposed as a realistic and achievable approach for preventing OA and thus reducing its burden on society. However, a greater understanding of the important roles of biomechanical factors, joint injury, obesity and metabolic syndrome in the pathogenesis of OA is required to reduce the effect of this disease on public health.

Glycolysis
An oxygen-independent metabolic pathway that generates two molecules of pyruvate, ATP and NADH from every one molecule of glucose, supporting the tricarboxylic acid cycle and providing intermediates for the pentose phosphate pathway, glycosylation reactions and the synthesis of biomolecules (including serine, glycine, alanine and acetyl-CoA).

Tricarboxylic acid (TCA) cycle
(Also known as the Krebs cycle) A set of connected pathways in the mitochondrial matrix, which metabolize acetyl-CoA derived from glycolysis or fatty acid oxidation, producing NADH and $FADH_2$ for the electron transport chain and precursors for amino acid and fatty acid synthesis.

Metabolism in articular cartilage

Articular cartilage is hypocellular, avascular, aneural and alymphatic[40]. Despite its hypocellularity, cartilage is a metabolically active connective tissue with reduced access to oxygen and glucose compared with synovial fluid and plasma[41], producing an environment that presents its few resident cells with a number of metabolic challenges. Consequently, the turnover of extracellular matrix (ECM) molecules in mature skeletal cartilage is extremely slow. For example, the turnover of proteoglycans (such as aggrecan) can take up to 25 years[42] and the half-life of type II collagen, the major fibrillary collagen in cartilage, is between 100 and 400 years[43,44]. Therefore, healthy cartilage with optimal ECM function requires the maintenance of a delicate balance between anabolic and catabolic activities, which is critical for long-term tissue integrity and the capacity for cartilage to repair itself[45].

Glucose is an important metabolic fuel and structural precursor in cartilage, being vital for ECM synthesis and degradation[46]. It serves as a major energy substrate as well as being the main precursor for the synthesis of glycosaminoglycans in chondrocytes[47] (FIG. 3). Facilitated glucose transport therefore represents the first rate-limiting step of glucose metabolism in chondrocytes[48], which express several glucose transporter isoforms, some of which are regulated by hypoxia and proinflammatory cytokines[41,48]. Once glucose is transported into chondrocytes, it is metabolized via glycolysis and the TCA cycle or used as a structural component for the synthesis of glycoproteins. Under normal regulatory homeostatic conditions and physiological normoxia (such as the oxygen environment in healthy cartilage), one molecule of glucose is oxidized by glycolysis, generating two molecules of pyruvate, which enter the mitochondria. Pyruvate is then decarboxylated by pyruvate dehydrogenase and enters the TCA cycle, producing $FADH_2$ and NADH, which donate electrons to the electron transport chain to generate 36 molecules of ATP per molecule of glucose by oxidative phosphorylation. Although chondrocytes rely primarily on glycolysis to meet their cellular energy requirements, they possess the metabolic flexibility to promote cell survival and support ECM biosynthesis during periods of nutrient stress by enhancing glycolysis and mitochondrial respiration through the TCA cycle[49]. Optimal mitochondrial function is therefore important for supporting the TCA cycle in healthy chondrocytes, and impaired mitochondrial function is implicated in OA pathogenesis[49].

Altered metabolism in OA

In pathophysiological situations such as OA, cellular metabolism is compromised and there is an increase in the production of antianabolic, procatabolic and proinflammatory factors[14]. The switch in metabolism that occurs to compensate for this compromised situation enables anabolic processes such as cell proliferation, protein biosynthesis, antigen presentation and phagocytosis still to occur in immune cells[3]. These anabolic processes equate to cell proliferation and protein biosynthesis in a variety of other cell types, including chondrocytes and other cells of the synovial joint. Indeed, emerging evidence suggests that in chondrocytes from patients with OA, proinflammatory pathways rely on energy generated by a metabolic switch from oxidative phosphorylation to glycolysis[50].

In healthy articular cartilage, chondrocytes have the metabolic flexibility to generate energy and promote cell survival during periods of acute nutrient stress by upregulating mitochondrial respiration and reducing the rate of reactive nitrogen and oxygen species production[49]. The metabolic adaptation of OA articular cartilage to new environmental conditions is evident in the early stages of the disease, when attempts to repair and regenerate the cartilage matrix have an increased likelihood of success[51]. However, cartilage from patients with OA at a later stage of disease does not seem to have this metabolic flexibility[51].

Cartilage and chondrocytes

The metabolic demands of fully differentiated and quiescent chondrocytes are very different from chondrocytes in an inflammatory microenvironment. Chondrocytes are highly glycolytic cells, which, like cancer cells, exhibit the 'Warburg effect' (also known as aerobic glycolysis)[52,53]. We do not yet understand the full molecular composition of the 'surfaceome' and 'membranome' of chondrocytes,

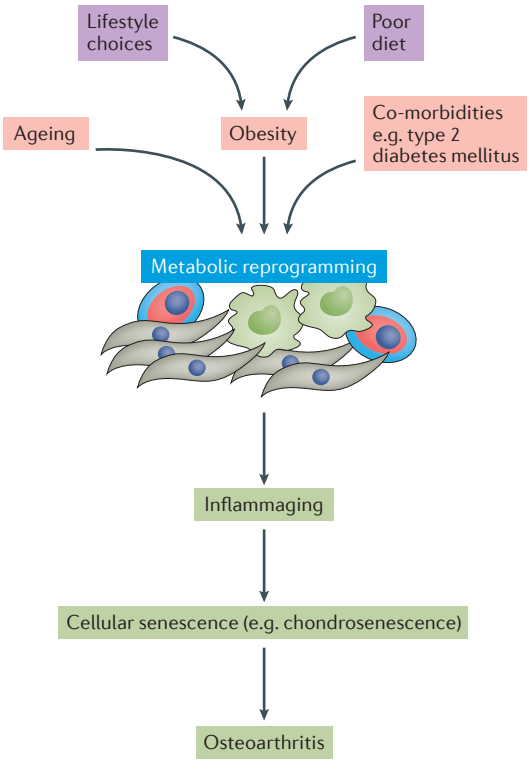

Figure 1 | **Factors underlying metabolic alterations in osteoarthritis.** Poor diet and lifestyle choices can contribute to weight gain and lead to obesity. Ageing, obesity and other co-morbidities associated with osteoarthritis (OA) contribute to metabolic reprogramming in a variety of cells and tissues, leading to inflammaging and cellular senescence, which in turn cause further changes in cellular metabolism in OA.

Pentose phosphate pathway
(PPP). An anabolic metabolic pathway parallel to glycolysis that branches out from glycolysis with the conversion of glucose-6-phosphate to ribose 5-phosphate and generates the reducing equivalents NADPH, ribose-5-phosphate (used in the synthesis of nucleotides and nucleic acids) and erythrose-4-phosphophosphate (used in the synthesis of amino acids).

Fatty acid oxidation
A metabolic process that produces ATP from the oxidation of acetyl-CoA derived from the mobilization of fatty acids.

Inflammaging
The low-grade proinflammatory phenotype that accompanies ageing.

Warburg effect
The high utilization of glycolysis by rapidly proliferating cells and the subsequent release of lactate into the extracellular milieu; a phenomenon first described by Otto Warburg.

Metabolic syndrome
The collective term used to describe the combination of type 2 diabetes mellitus, high blood pressure, dyslipidemia and obesity.

Electron transport chain
A series of proteins in the inner mitochondrial membrane that transfer electrons from one to the other in a series of redox reactions, resulting in the movement of protons out of the mitochondrial matrix and in the synthesis of ATP.

Oxidative phosphorylation
A metabolic pathway that produces ATP from the oxidation of acetyl-CoA and the transfer of electrons to the electron transport chain via NADH and $FADH_2$.

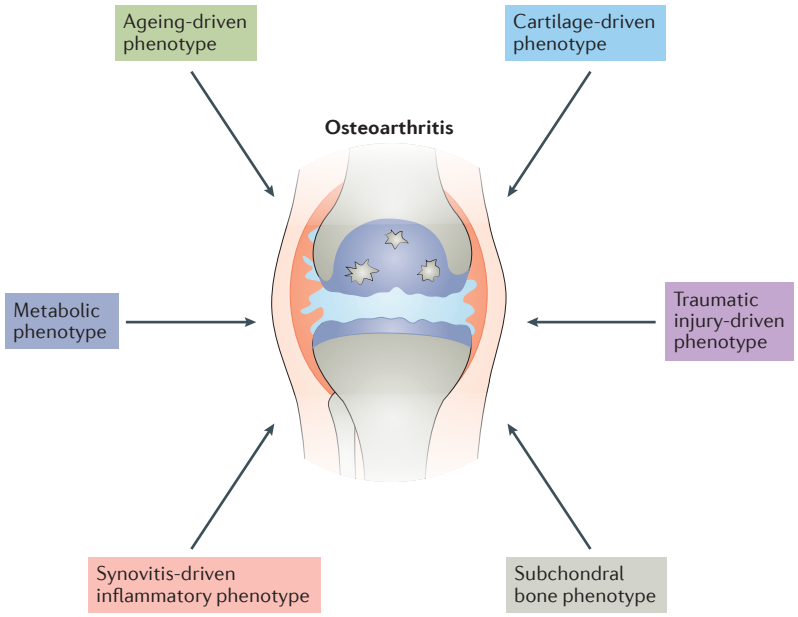

Figure 2 | **Phenotypes of osteoarthritis.** Evidence suggests that patients with osteoarthritis (OA) fall into multiple phenotypic subgroups defined on the basis of the main driver of disease, one of which is a distinct metabolic phenotype, although all OA phenotypes probably involve metabolic alterations. Cartilage, bone and synovium are all affected by external and internal drivers of disease such as inflammation, injury or biomechanical alterations, metabolic reprogramming and immunomodulation, but different synovial joint tissues dominate the disease in different patients with OA.

but proteomic studies have revealed that metabolic adaptation of chondrocytes to an inflammatory microenvironment has a direct effect on the composition of membrane proteins in these cells (A.M., unpublished data). Chondrocytes are also able to sense the concentrations of oxygen and glucose in the ECM and respond appropriately by adjusting their cellular metabolism, thus becoming glycolytic during periods of acute nutrient and oxygen stress[41].

Glycolytic pathways. The metabolic switch in activated chondrocytes means that these cells derive ATP from glycolysis, diverting pyruvate away from oxidative phosphorylation and thereby enabling ATP generation during periods of low oxygen availability (FIG. 4). Chondrocytes express the glucose transporter GLUT1 (REF. 54), which is upregulated in response to hypoxia[55], thus increasing their ability to take up glucose in low oxygen conditions. Levels of glucose-6-phosphate dehydrogenase are also increased in cartilage explants exposed to oxidative damage[56], indicating increased glycolytic activity. Anaerobic glycolysis occurs at an increased rate in chondrocytes in OA[57]; lactate dehydrogenase converts two pyruvate molecules into lactate in the cytosol, generating two molecules of ATP (instead of the 36 molecules generated by oxidative phosphorylation) and leading to an accumulation of lactate, further reducing the pH of an already acidic microenvironment[58]. Profound ATP depletion in chondrocytes is associated with increased production of nitric oxide (NO) in the osteoarthritic joint[49]. Consequently, activity of ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (an ATP scavenger) increases, causing a subsequent increase in extracellular levels of inorganic pyrophosphate. This increase in inorganic pyrophosphate might in turn stimulate chondrocalcinosis, eventually leading to matrix calcification (an important contributor to OA progression[59]). It should be noted, however, that the ubiquitous presence of calcification in patients with OA identified in a CT imaging study[60] has raised questions over the appropriateness of the term 'chondrocalcinosis'.

Mitochondria and oxidative stress. Mitochondria are the powerhouses of the cell, providing energy in the form of ATP for a range of activities including movement, cellular differentiation, cell death, regulation of signalling and control of the cell cycle[61]. Furthermore, mitochondria serve as molecular platforms integrating multiple innate immune signalling pathways[62]. However, in degenerative conditions (such as OA), alterations occur in mitochondrial structure, dynamics and genome stability, resulting in reduced mitochondrial respiration and excessive production of reactive oxygen species (ROS) leading to oxidative damage. Mitochondrial dysfunction and oxidative stress are hallmarks of OA[63], with increased mitochondrial DNA (mtDNA) damage being seen in chondrocytes from patients with OA compared with chondrocytes from healthy individuals[64]. This damage in chondrocytes from patients with OA is accompanied by a reduced capacity for mtDNA repair and an increased rate of apoptosis[64].

Alterations in mitochondrial membrane potential are observed in chondrocytes from patients with OA[65]. Analysis of mitochondrial electron transport chain activity in chondrocytes from patients with OA has shown a decrease in complexes II and III compared with chondrocytes from healthy individuals, along with a reduction in mitochondrial membrane potential[57] (maintenance of mitochondrial membrane potential is essential to driving ATP synthesis by oxidative phosphorylation). Although most ATP in chondrocytes in OA comes from glycolysis rather than oxidative phosphorylation[66], mitochondrial ROS help to maintain the cellular redox balance in favour of glycolysis[67]. Treatment of chondrocytes from patients with OA with 4-hydroxynonenal (4HNE) (an end-product of lipid peroxidation and a second messenger in oxidative stress) results in the depletion of ATP, NADPH and glutathione and the inhibition of glucose uptake and TCA cycle activity[68]. Additionally, inhibition of complexes III and V of the electron transport chain modulates the expression of matrix metalloproteinases (MMPs) in chondrocytes and proteoglycan levels in cartilage[69]. Therefore, the loss of energy reserves within chondrocytes coupled with a shift in metabolic pathways towards glycolysis contributes to the impaired ECM synthetic function, anabolism and reduced viability seen in chondrocytes in OA[69,70]. Furthermore, NADPH oxidase 4 is increased in chondrocytes from patients with OA and can modulate matrix degrading enzymes

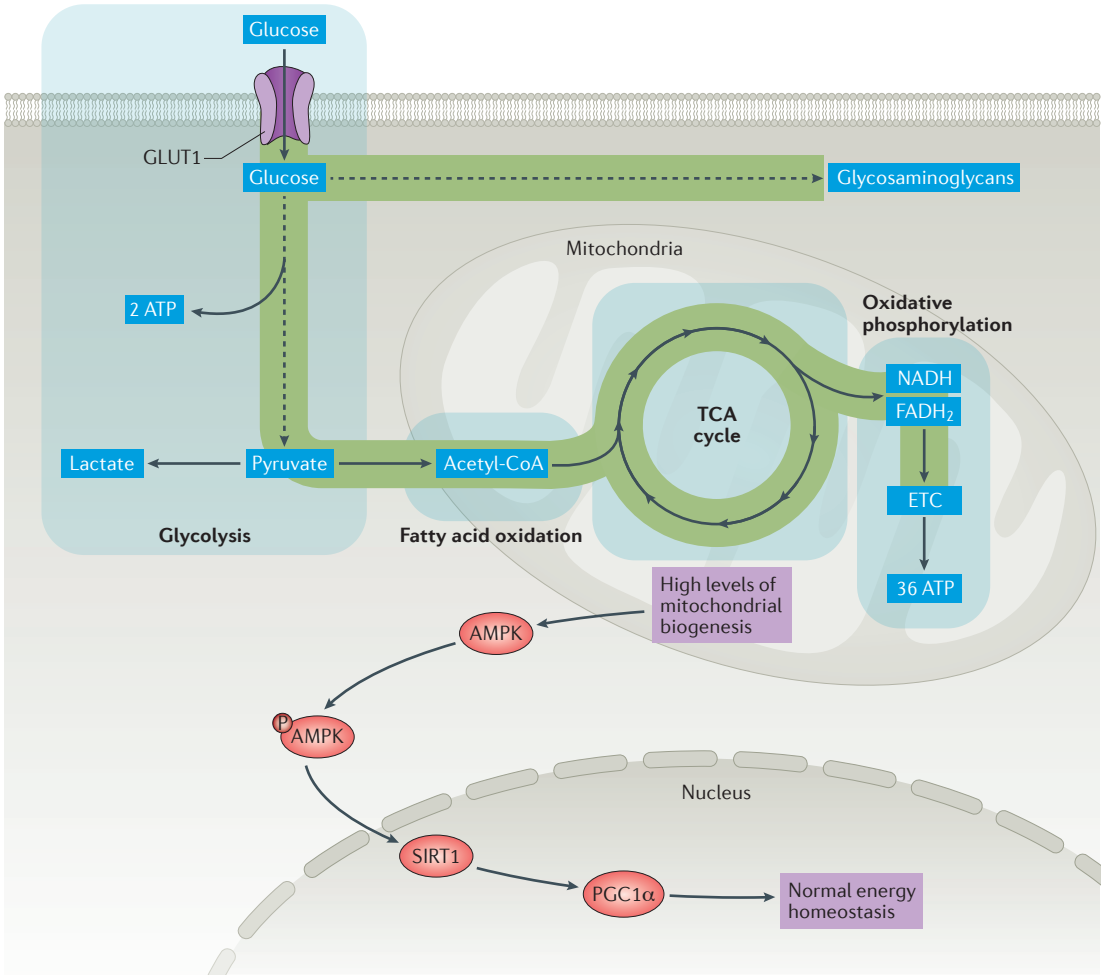

Figure 3 | **Metabolism in homeostatic chondrocytes.** In healthy joints, chondrocytes utilize glucose as well as other metabolic fuels and sources of energy. Glucose utilization via glycolysis and oxidative phosphorylation helps to maintain an optimal level of mitochondrial function and biogenesis. The metabolism of healthy chondrocytes is therefore optimized to maintain normal energy homeostasis via signalling through the AMPK–SIRT1–PGC1α pathway. AMPK, AMP-activated protein kinase; ETC, electron transport chain; GLUT1, glucose transporter type 1; PGC1α, peroxisome proliferator-activated receptor γ co-activator 1α; ROS, reactive oxygen species; SIRT1, NAD-dependent protein deacetylase sirtuin-1; TCA, tricarboxylic acid.

such as MMPs and the a disintegrin and metalloproteinase with thrombospondin motif (ADAMTS) family of proteins by inducing ROS production[71].

Oxidative stress mediated by ROS and NO also has an important role in metabolic dysregulation within the osteoarthritic joint[72–74]. NO production is elevated in human OA, animal models of spontaneous OA, animal models of experimentally-induced OA and in cytokine-treated or activated chondrocytes[75–77]. Furthermore, inducible nitric oxide synthase (iNOS) is present in both the synovium and cartilage, and its expression has been observed in degenerating regions of OA cartilage[76,77]. NO and other ROS are involved in the accumulation of mtDNA damage following exposure to cytokines; the mitochondria-targeted DNA repair enzyme hOGG1 was able to rescue mtDNA integrity, preserve ATP levels, re-establish mitochondrial transcription and diminish apoptosis in chondrocytes following exposure to IL-1β and TNF[78]. Studies in Kashin–Beck disease (KBD), a chronic and endemic osteochondropathy prevalent mainly in Tibet and China, have also increased our understanding of the role of metabolism in osteoarticular disease. In KBD, chondrocytes exhibit increased numbers of de-energized mitochondria, a reduction in cellular ATP levels and an increase in mitochondrial mass, release of cytochrome *c* (a key component of the electron transport chain in mitochondria) and activation of caspase 9 and caspase 3, leading ultimately to cell apoptosis[79]. Markers of oxidative stress are also higher in cartilage from patients with KBD than in cartilage from healthy individuals[80].

Increased levels of lipid peroxidation, as found in the osteoarthritic joint, also lead to an increase in breaks in mtDNA in chondrocytes from patients with OA, which in turn affects the telomeric DNA and replicative lifespan of chondrocytes, as well as the subsequent integrity of proteoglycans in osteoarthritic cartilage[45,81]. The adverse microenvironment of the osteoarthritic joint leads to the

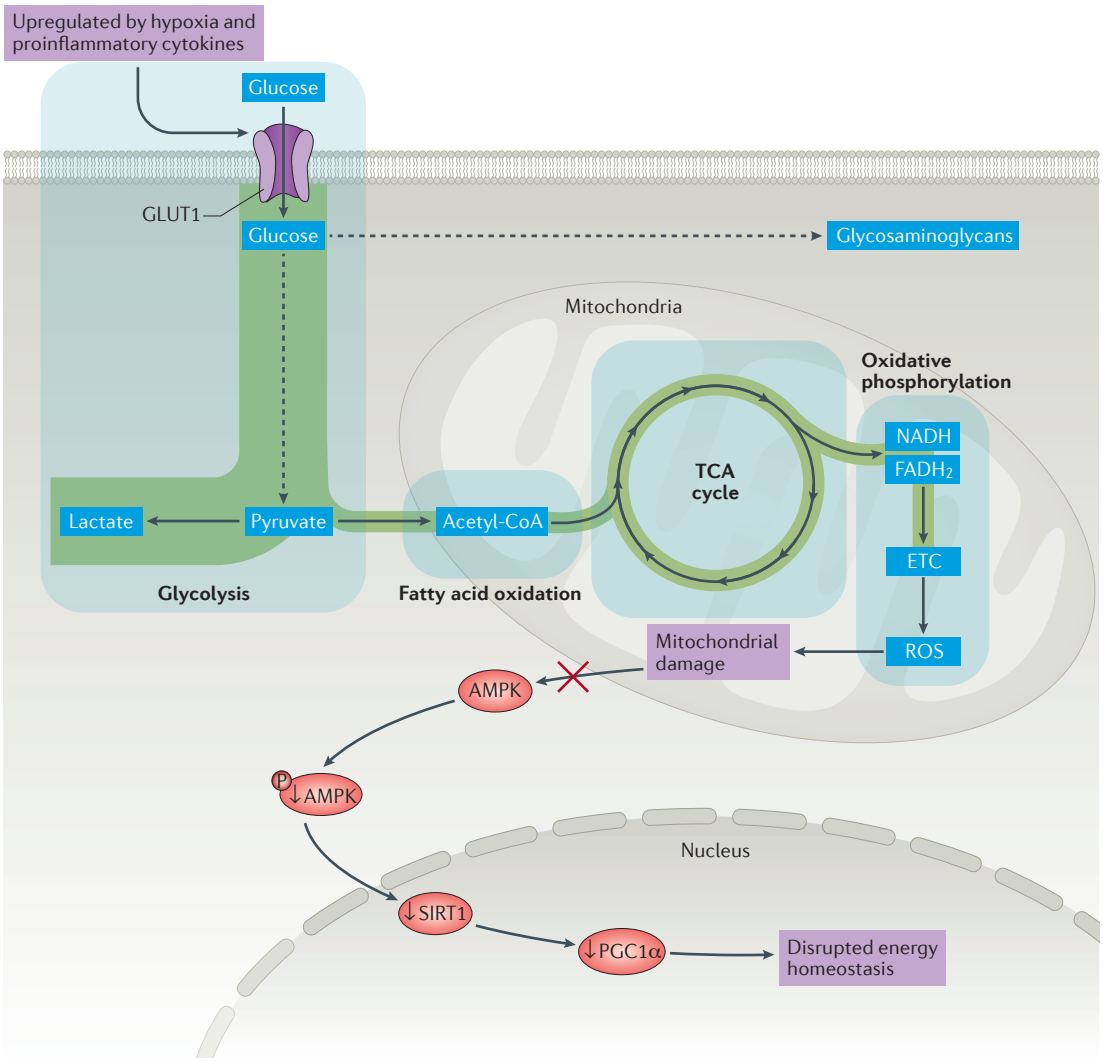

Figure 4 | **Altered metabolism in chondrocytes in osteoarthritis.** Chondrocytes in osteoarthritis (OA) switch from oxidative phosphorylation to glycolysis as their main source of energy metabolism. In osteoarthritic joints, chondrocytes are exposed to proinflammatory cytokines and microenvironmental alterations, including hypoxia and nutrient stress. Mitochondrial metabolism is impaired and reactive oxygen species (ROS) accumulate, causing damage to mitochondria which inhibits AMPK signalling and activity, downregulate SIRT1 and decrease levels of PGC1α, the master regulator of mitochondrial biogenesis. AMPK, AMP-activated protein kinase; ETC, electron transport chain; GLUT1, glucose transporter type 1; PGC1α, peroxisome proliferator-activated receptor γ co-activator 1α; SIRT1, NAD-dependent protein deacetylase sirtuin-1; TCA, tricarboxylic acid.

increased generation of ROS and NO by chondrocytes[82] (and synoviocytes[83]), inducing more mtDNA damage and suppressing mitochondrial oxidative phosphorylation[69,84,85], acting as a feed-forward loop. These changes increase the production of MMPs[85–87] and can enhance the responsiveness of chondrocytes to cytokine-induced inflammation through nuclear factor-κB activation[87]. ROS scavengers slow down cartilage loss in animal models of joint inflammation[88] and decrease levels of MMPs in chondrocytes[85,89].

Key regulators of metabolism. A key molecule associated with metabolism in chondrocytes is AMP-activated protein kinase (AMPK), which regulates energy metabolism through the downstream mediators, NAD-dependent protein deacetylase sirtuin-1 (SIRT1) and mechanistic target of rapamycin (mTOR). Depletion of AMPK in chondrocytes increases their catabolic response to proinflammatory cytokines[90]. A decreased capacity for mitochondrial biogenesis in chondrocytes is linked to reduced AMPK activity and decreased expression of SIRT1, peroxisome proliferator-activated receptor γ co-activator 1α (PGC1α; the so-called master regulator of mitochondrial biogenesis), TFAM (transcription factor A, mitochondrial), nuclear respiratory factor 1 (NRF1) and NRF2 (REF. 91). TFAM-mediated activation of the AMPK–SIRT1–PGC1α pathway increases mitochondrial biogenesis in chondrocytes, limiting OA progression[91]. Furthermore, deficiency in AMPK and SIRT1 modulates PGC1α activity, leading to reduced oxidative

stress and procatabolic responses in chondrocytes from patients with OA[91], and so potentially represents a mechanism to inhibit the progression of cartilage damage in OA[92]. Inhibition of SIRT1 results in increased procatabolic responses to IL-1β and TNF in chondrocytes from patients with OA[93,94]. Additionally, autophagy, which is known to have chrondroprotective effects but is defective in chondrocytes from patients with OA, is promoted by AMPK and SIRT1, resulting in the subsequent repair of damaged mitochondria[95].

In the destabilisation of medial meniscus (DMM) mouse model of OA, cartilage-specific deletion of mTOR upregulated autophagy and protected mice from disease[96], whereas aberrant mTOR signalling associated with peroxisome proliferator-activated receptor γ deficiency resulted in severe and accelerated OA[97]. Cell survival and matrix synthesis is suppressed in chondrocytes from patients with OA via microRNA 634 targeting of *PIK3R1*, thus modulating the PI3K–AKT–ribosomal protein S6 kinase (S6) and PI3K–AKT–mTOR–S6 signalling pathways[98]. Many other regulatory molecules are also implicated in chondrocyte physiology and pathophysiology and have links to metabolism[5,99–101], but the overt effects of activation and inhibition of these pathways on cellular metabolism are not yet understood.

Chondrosenescence. Once activated by stressors such as proinflammatory cytokines, prostaglandins and ROS, normally quiescent articular chondrocytes become activated and undergo a phenotypic shift through a phenomenon recently described as 'chondrosenescence', leading to further disruption of homeostasis and metabolism in cartilage[6]. The chondrosenescent phenotype is highly procatabolic and is intimately linked with a disturbed interplay between autophagy and inflammasomes[102], and with the development of a senescent secretory and inflammatory state[6]. The production and secretion of soluble and insoluble factors by senescent chondrocytes further contributes to the inflammatory microenvironment that is believed to drive the catabolic degradation of ECM macromolecules in articular cartilage. Furthermore, the secreted molecules, in particular NO, act as potent inducers of gene expression, further supporting the aberrant expression of proinflammatory and catabolic genes. These secreted molecules also suppress mitochondrial dysfunction and impair oxidative phosphorylation[103] which in turn can promote calcification of the ECM and formation of inflammatory hydroxyapatite crystals[69,84].

Synovium

In addition to cartilage, other joint tissues such as the synovial membrane[104,105], subchondral bone[106] and periarticular soft tissues[107] contribute to the disease process in OA. Inflammation of the synovium occurs in early and late phases of OA and is associated with degenerative alterations in cartilage[108]. This synovitis is qualitatively and histologically similar, but not identical, to that seen in patients with RA. Despite some similarities, there are important differences too; notably, the polymorphonuclear leukocytes that are prominent in synovitis in RA are absent in synovitis in OA[109]. Synovitis linked to the innate immune system[59] has a key role in OA pathogenesis and influences metabolism in joint tissues[108]. Catabolic and proinflammatory mediators produced by the inflamed synovium such as cytokines, ROS, NO, prostaglandin E_2 and neuropeptides alter cellular metabolism and the balance of cartilage matrix degradation and repair[110]. Synovitis seems to be a common feature of later stages of OA, which are characterized by infiltrating macrophages, T cells and mast cells and high levels of proinflammatory cytokines[111]. Inflammation of the synovium leads to increased production of the proteolytic enzymes responsible for cartilage breakdown[112]. Increased catabolism in cartilage releases molecules that induce further synovial inflammation, creating a feed-forward loop that exacerbates clinical symptoms and joint degradation in patients with OA[31]. Inflammatory mediators released by chondrocytes and synoviocytes also drive oxidative stress, causing damage to joint tissues via ROS[113].

Immune cells. Evidence for metabolic changes in immune cells in the inflamed osteoarthritic joint is scarce and indirect. There is evidence from metabolomics studies for metabolic changes in immune cells in OA. Metabolic profiling has identified changes in metabolites specific to collagen metabolism, branched-chain amino acid metabolism, energy metabolism and tryptophan metabolism in OA, suggesting that the metabolic state alters as the disease progresses[114]. Metabolomics is particularly well suited for OA research because of the tremendous heterogeneity in the disease process and recognition that no single biomarker can reflect the breadth of temporal and pathological processes involved.

Fibroblast-like synoviocytes. In contrast to the metabolic profile of chondrocytes in OA, which has been extensively studied, little is known about the metabolic profile of FLSs and immune cells that infiltrate the synovium in patients with OA. To date, the majority of studies have focused on FLSs in RA, in which a hypoxia-induced shift towards glycolysis is associated with increased migration and invasiveness[95,115]. Chronic hypoxia alters cellular bioenergetics by inducing mitochondrial dysfunction and glycolytic pathways, thereby supporting abnormal angiogenesis, cellular invasion and pannus formation in the joints of patients with RA[115]. Glucose metabolism therefore has a critical role in the activity and behaviour of FLSs in RA[116]. The glycolytic enzyme glucose-6-phosphate isomerase (GPI) also promotes the proliferation of and inhibits the apoptosis of FLSs from patients with RA[117]. Interestingly, GPI is a multifunctional protein that also acts as an angiogenic factor to stimulate endothelial cell motility[118]. Upregulation of glucose transport and a switch to glycolysis have been implicated in the regulation of angiogenesis in RA and OA[119].

In a 2016 study, an increase in the ratio of glycolysis to oxidative phosphorylation was observed in FLSs, although this increase was lower in FLSs from patients with OA than in those from patients with RA[115]. Similar levels of the glucose metabolism-related genes *LDHA* and

PDHK1 were seen in FLS from patients with OA and RA; however, expression of *GLUT1* and *HK2* were increased only in FLSs from patients with RA[115]. Following stimulation with lipopolysaccharide, GLUT1 expression and lactate levels were increased in FLSs from patients with OA, and blockade of glycolysis inhibited the migratory capacity of these cells[115]. In another study, administration of high concentrations of glucose to FLSs from patients with OA induced the expression of vascular endothelial growth factor (VEGF) and the production of ROS via the PIK3–ATK signalling pathway[120]. Blockade of the universal oxygen sensor prolyl hydroxylase domain-containing protein 2 (PHD2; also known as Egl nine homologue 1) in FLSs from patients with OA increased the expression of angiogenic factors, which were subsequently able to induced tube formation by endothelial cells[121]. Connective tissue growth factor-induced IL-1β expression in FLSs from patients with OA is mediated by αvβ3/αvβ5 integrin-dependent generation of ROS, the blocking of which with berberine prevented cartilage damage in a rat model of OA[122]. Furthermore, oxidative stress induces prostaglandin G/H synthase 2 (also known as COX2) expression in FLSs from patients with OA, an outcome that can be reversed by the antioxidant N-acetyl cysteine[123]. Collectively, these studies highlight a critical role for glucose transport and metabolism in FLSs in the synovium of patients with OA, as well as in patients with RA[116].

Metabolic targets in OA therapy?

Several studies have reported the consequences of blocking metabolic regulators such as AMPK and mTOR in *in vitro* and *in vivo* models of OA. Intra-articular injection of rapamycin (which targets mTOR) into mice with experimental OA substantially reduced the severity of damage to articular cartilage, an effect mediated by an increase in autophagy and by inhibiting the production of VEGF, collagen type X α1 chain and MMP13 (REF. 124). In another study using a mouse model of OA, treatment of mice with rapamycin reduced the severity of cartilage degradation and synovitis, an effect that was accompanied by a decrease in the expression of ADAMTS5 and IL-1β in articular cartilage[125]. Taken together, these studies suggest that pharmacological activation of autophagy via mTOR signalling pathways might be an effective therapeutic approach for treating OA. Decreased AMPK activity is also associated with cartilage damage; chondrocytes from patients with OA that have been depleted of AMPK exhibit increased catabolic responses to proinflammatory cytokines and biochemical injury, effects that are attenuated by molecules thought to activate AMPK[126,127]. Thus AMPK-activating drugs such as methotrexate, metformin and sodium salicylate could have therapeutic effects in this disease[127].

The glycolytic switch also represents a potential therapeutic target in arthritis. Inhibition of glycolysis might seem counterintuitive, but modulation of glycolytic pathways could directly modulate the responses of FLSs and chondrocytes to inflammatory mediators, thereby making inhibition of glycolysis a potentially effective treatment strategy for OA and RA. Specifically, overexpression of the key glycolytic enzyme 6-phosphofructo 2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) (the activity of which is impaired in cartilage in OA) reduces the activity of caspase 3 and promotes the production of aggrecan and type II collagen in explants of cartilage and chondrocytes from patients with OA[128]. These results indicate that PFKFB3 might also provide a therapeutic target for the treatment of OA. A more detailed understanding of the molecules that regulate these metabolic switches could enhance the efficacy of biological therapies for patients with RA so that treatments can be more rationally applied and personalized for patients. There are currently no such treatments for OA[129], but increasing our knowledge of metabolism within the joint could reveal mechanistic insights necessary for the development of new therapies for OA. Therapeutically targeting metabolic pathways to treat rheumatic diseases is covered in depth in a Perspectives article[130] in this journal.

Conclusions

The pathogenesis of OA involves metabolic alterations in articular cartilage, subchondral bone and synovium. These changes influence metabolic pathways in chondrocytes, synoviocytes and bone cells and their interactions with the immune system via inflammatory mediators. Accumulating evidence suggests that a metabolic switch towards glycolysis is important for immune responses and the activation of inflammatory pathways in chronic diseases, including OA and RA. This change in metabolism enables immune and inflammatory cells to gain energy to meet the increased demands for the biosynthesis of proinflammatory and degradative proteins during periods of acute cellular stress or nutrient deprivation. Similar mechanisms seem to operate in cells of the synovial joint in OA. A deeper mechanistic understanding of these complex metabolic pathways is therefore likely to provide insight into potential novel therapeutic strategies for treating OA and other inflammatory diseases of joints. At this point, research into immunometabolism in OA is still in its infancy; however, if the availability of glucose and oxygen are impaired in immune cells in OA, then regulators such as mTOR, AMPK and hypoxia-inducible factor 1α represent potential starting points for the discovery of therapeutic targets. An improved understanding of physiologic and pathophysiologic regulators of cartilage and synovial metabolism is also likely to provide new insights into the aetiology and pathophysiology of OA. Omics techniques such as metabolomics are likely to identify some of the underlying metabolic changes in OA[131] and help to define the metabolic phenotype of OA[132,133], especially in the early stages of disease[134]. When combined with proteomics, lipidomics and bioinformatics, metabolomics will help to reveal the pathways, proteins and metabolites that drive inflammatory processes in synovial joints, hopefully also revealing new therapeutic targets. Future research should also focus on delineating the role of metabolism in macrophages that infiltrate the synovium in OA and in FLS in OA.

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Review criteria
We searched the MEDLINE and PubMed databases for original articles on immunometabolism published before February 2017, using the search terms "inflammation", "mitochondria", "inflammasome", "cytokine", "immunometabolism", "osteoarthritis", "articular cartilage", "chondrocyte", "synovitis", "synovium", "synoviocyte", "macrophage", "homeostasis", "ageing", "glycolysis", "oxidative phosphorylation", "cell signalling", "nutrient", "glucose", "oxygen", "adipokine", "adiposity", "diabesity". All articles identified for inclusion in the review were full-text English-language articles or reviews. We also searched the reference lists of the identified manuscripts for additional relevant articles.

PERSPECTIVES

OPINION

Fine tuning of immunometabolism for the treatment of rheumatic diseases

Jillian P. Rhoads, Amy S. Major and Jeffrey C. Rathmell

Abstract | All immune cells depend on specific and efficient metabolic pathways to mount an appropriate response. Over the past decade, the field of immunometabolism has expanded our understanding of the various means by which cells modulate metabolism to achieve the effector functions necessary to fight infection or maintain homeostasis. Harnessing these metabolic pathways to manipulate inappropriate immune responses as a therapeutic strategy in cancer and autoimmunity has received increasing scrutiny by the scientific community. Fine tuning immunometabolism to provide the desired response, or prevent a deleterious response, is an attractive alternative to chemotherapy or overt immunosuppression. The various metabolic pathways used by immune cells in rheumatoid arthritis, systemic lupus erythematosus and osteoarthritis offer numerous opportunities for selective targeting of specific immune cell subsets to manipulate cellular metabolism for therapeutic benefit in these rheumatologic diseases.

Inflammatory and autoimmune diseases are driven by the activation and effector functions of both innate and adaptive immune cells. In addition to neutrophils and other cells involved in acute inflammation, macrophages and dendritic cells are activated to promote T and B lymphocyte responses in rheumatologic diseases such as systemic lupus erythematosus (SLE)[1] and rheumatoid arthritis (RA)[2]. Osteoarthritis (OA), although generally considered non-inflammatory, can present with an inflammatory phenotype and the inflammatory processes involved in this disease are increasingly recognized[3]. In each of these diseases, inflammatory cytokines stimulate immune cells[4] or monocyte-to-osteoclast differentiation to promote autoimmunity or bone resorption and degradation[5,6]. Although these rheumatologic diseases have unique characteristics, in each setting haematopoietic cells must be stimulated to gain effector functions and differentiate. The signalling and gene expression changes that accompany these cellular activation and differentiation events have been well studied, but it is now apparent that the metabolism of disease-effector cells is also tightly regulated[6–9]. Each inflammatory cell, and even anti-inflammatory cell, undergoes metabolic reprogramming upon activation and these changes are essential for disease. Therefore, targeting the metabolic pathways involved offers a new avenue for potential treatment of rheumatologic diseases. Because immunological functions are associated with specific metabolic programmes, this approach affords the particularly attractive possibility that inhibiting the appropriate pathway could lead to selective, cell-specific blockade. In this Perspectives article, we discuss the various metabolic pathways used by immune cells to attain optimal responses and explore the possibility and key principles of manipulating these pathways for therapeutic benefit in rheumatologic diseases, with a focus on RA, SLE and OA.

Cellular metabolic reprogramming

Activation of immune cells leads to changes in metabolic pathways. Resting lymphocytes, macrophages and dendritic cells all use catabolic metabolic pathways that switch to anabolic programmes after activation by antigens, cytokines or stimulation of innate pattern-recognition receptors by pathogen-associated or damage-associated molecular patterns (PAMPs and DAMPs, respectively)[7]. This switch supports resting cell survival and immune surveillance as well as growth and effector function of stimulated cells. Resting T cells take up glucose, amino acids and lipids at a low rate and flux these fuels through glycolysis, glutaminolysis and fatty acid oxidation to maximize mitochondrial oxidative metabolism[7]. This mode of metabolism generates maximal ATP and is associated with a long T cell lifespan[10–13]. Given the need to maintain osmolarity through the sodium–potassium ATPase and the energy demands of rapid chemotaxis and cytoskeletal remodelling during this surveillance mode of resting lymphocytes[14], it is not surprising that metabolism in resting immune cells is programmed to actively support the most efficient ATP-generating processes.

Lymphocyte stimulation leads to abrupt changes in metabolic pathways in these cells. Stimulation of T cells through the T cell receptor in conjunction with co-stimulation leads to a sharp increase in glycolysis and glutaminolysis[15–17] (FIG. 1). Simultaneously, activated T cells decrease mitochondrial fatty acid oxidation in order to conserve lipids for new membrane synthesis[18,19]. Co-stimulatory signals have key roles in this transition; CD28 augments glucose uptake and glycolysis in activated T cells[16], whereas inhibitory receptors, such as cytotoxic T-lymphocyte protein 4 (CTLA4) and programmed cell death protein 1 (PD-1), can decrease glycolysis and instead promote mitochondrial fatty acid oxidation[15,20–22]. In part, these regulators act through control of signalling via phosphatidyl-inositol 3-kinase (PI3K), AKT and mechanistic target of rapamycin (mTOR)[23]. Resting B cells undergo a similar metabolic shift upon activation. Stimulation of B cells through antigen receptors or Toll-like receptors (TLRs) leads to upregulation of the glucose

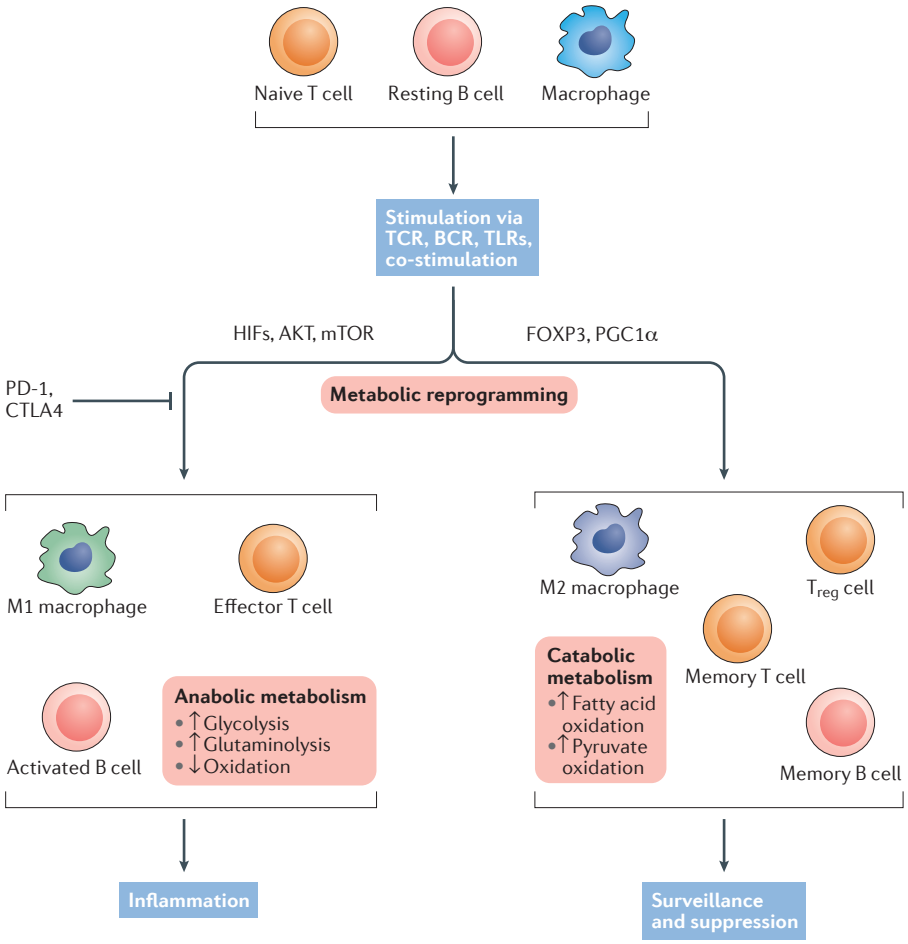

Figure 1 | **Metabolic reprogramming of immune cell populations matches immunological function.** Naive T cells, resting B cells and macrophages utilize a catabolic and oxidative metabolic programme. After stimulation via antigen receptor with co-stimulation or through pattern-recognition receptors such as Toll-like receptors (TLRs), these immune cells undergo metabolic reprogramming. Effector lymphocytes or inflammatory macrophages induce an anabolic metabolic programme with highly increased nutrient uptake for glycolysis and glutamine metabolism. Regulatory cells or alternatively activated macrophages, by contrast, primarily utilize a programme of lipid and pyruvate oxidation. These programmes are important to the function of each subset; if the cellular metabolism does not match the cell fate, immune cells will fail to gain appropriate functional capacity. BCR, B cell receptor; CTLA-4, cytotoxic T lymphocyte protein 4; HIF, hypoxia-inducible factor; mTOR, mechanistic target of rapamycin; PD-1, programmed cell death protein 1; PGC1α, peroxisome proliferator-activated receptor γ co-activator 1-α; TCR, T cell receptor; T_{reg} cell, regulatory T cell.

transporter GLUT1 and glycolysis[24,25]. As in T cells, this metabolic reprogramming is dependent on mTOR signalling, as deficiency of regulatory-associated protein of mTOR (RAPTOR) and mTOR complex I (mTORC1) or alteration of the PI3K pathway disrupts B cell development and activation, and can impair class-switching in germinal centres[26–28]. Ultimately, as an immune response ceases, memory lymphocytes revert to oxidative pathways that are essential to enabling persistence of memory and robust secondary responses[10,12]. Memory lymphocytes can, however, retain enhanced metabolic features that facilitate rapid and strong secondary responses[29,30].

Dendritic cells and macrophages differ from lymphocytes in that proliferation is not as important a cellular goal following activation. The ability to mature and gain effector function (including the differentiation of monocytes into osteoclasts) is, however, essential for these cells. Macrophages and dendritic cells are activated in response to PAMPs and DAMPs, including TLRs, and this activation increases glycolysis to promote inflammatory function and maturation[31–35]. TLR signalling through serine/threonine-protein kinase TBK1 leads to AKT activation and mTORC1 signalling to promote this glycolytic switch[36–38].

Increased glycolysis both promotes inflammation and can enhance 'trained immunity', a process that, although not specific in the same way as adaptive immune responses, can lead to improved secondary innate responses[32,39]. In addition to enabling enhanced biosynthesis of effector molecules and cytokines, this metabolic reprogramming supports the growth of essential cell structures, such as the endoplasmic reticulum and Golgi[37], which have critical roles in the cell biology of effector function.

Metabolic programmes are specific for immune cell subsets and functions. A critical aspect of the metabolic reprogramming events described above is that they are not uniform in a given cell type, but instead utilize specific pathways that are essential for particular cell subsets and functions (FIG. 1). This specificity was first demonstrated in classical 'M1' macrophages and alternatively activated 'M2' macrophages, in which activation with IL-4 led to a peroxisome proliferator-activated receptor γ co-activator 1-β (PGC1β)-dependent increase in oxidative metabolism that contrasted with the more glycolytic metabolism of macrophages activated by IFNγ and the TLR4 ligand lipopolysaccharide[40]. Indeed, these metabolic pathways were linked to the functions of the cells, as promoting increased glucose uptake by GLUT1 expression enhanced proinflammatory macrophage activity[35], whereas promoting mitochondrial lipid oxidative pathways stimulated anti-inflammatory macrophage function[40,41].

Although OA is characterized by subchondral sclerosis[42], inflammation and innate immunity can contribute substantially to disease pathogenesis[3]. The differentiation of monocytes/macrophages into osteoclasts that contribute to inflammation in OA also depends on specific metabolic programmes. In particular, hypoxia and the hypoxia-inducible factors HIF-1α and HIF-2α promote osteoclast differentiation[43,44]. Increased lactic acid, which can suppress glycolysis, also promotes generation of osteoclasts[45]. Together, these findings support the model that different macrophage-derived subsets have distinct metabolic programmes that promote, and are intimately linked to, cell function and fate.

Subsequent to these early studies in macrophages, T cell subsets were also found to utilize distinct metabolic programmes[46],

with particular differences noted between regulatory T (T_{reg}) cells and $CD4^+$ effector subsets, including type 1 T helper (T_H1), T_H2, and T_H17 cells[19,46,47]. Effector T cells are largely glycolytic downstream of mTOR signals[48] that differentially affect specific CD4 subsets through mTORC1 or mTORC2 (REF. 49), whereas T_{reg} cells preferentially utilize a mitochondrial oxidative metabolism consisting of lipid and pyruvate oxidation[19,46,47]. Indeed, whereas lipid synthesis is required for T_H17 cells, and overproduction of lipids can lead to T cell phenotypes associated with autoimmunity[50], lipid oxidation promotes T_{reg} cell differentiation[51]. This alternative metabolic programme is regulated by the T_{reg} cell transcription factor FOXP3 (REFS 52,53) as well as by PGC1α and hSIRT3 (also known as NAD-dependent protein deacetylase sirtuin-3, mitochondrial)[54]. *In vivo*, effector T cells depend on GLUT1 (REF. 55) as well as the amino acid transporters solute carrier family 1 member 5 (SLC1A5, also known as ASCT2 or neutral amino acid transporter B(0))[56] and solute carrier family 7 member 5 (SLC7A5, also known as large neutral amino acids transporter small subunit 1 or LAT1)[57], whereas T_{reg} cells can function independently of these transporters[55–57]. T_{reg} cells can, however, initiate glycolysis, in a manner dependent on mTORC1 activation for proliferation[58–60] following activating or inflammatory signals[52]. Increased glycolysis in T_{reg} cells augmented proliferation but also reduced the suppressive capacity of these cells[52]. This switch between maximal T_{reg} cell proliferation or suppressive capacity was controlled in part by the PI3K–AKT–mTORC1 pathway, and constitutive activation of AKT or mTORC1 led to accumulation of poorly suppressive T_{reg} with low phenotypic stability[52,61–63]. Tight regulation of mTOR activity is thus required for T_{reg} cell function. In other $CD4^+$ T cell subsets, such as T follicular helper cells, metabolism seems to be more balanced and relies on both glycolysis and oxidative phosphorylation[64,65]. Metabolism in macrophages and dendritic cells is also regulated by mTORC1 and mTORC2 signalling[66,67]. In particular, signalling through mTORC1 can promote glycolysis, which can enhance M1 macrophage activation[35,41,66], whereas M2 macrophages utilize oxidative metabolism that is regulated by signal transducer and activator of transcription 6 (STAT6) and PGC1β[40]. Inhibition of mTOR kinase can, therefore, alter macrophage metabolism and might affect macrophage subsets.

Immunometabolism in disease

Chronic encounters with autoantigens and inflammatory signals can sharply alter immunometabolism in ways that differ from the response to acute stimulation. Indeed, chronic viral infections diminished glucose metabolism in T cells[68]. Alterations in immunometabolism in inflammatory diseases reveals insight into disease processes and potential therapeutic targets.

Systemic lupus erythematosus. Metabolomics analyses of sera from patients with SLE have revealed a variety of considerable alterations in metabolites and metabolic pathways that correlate with disease activity and manifestations[69–71]. Although serum metabolites can be affected by multiple cell types and tissues, several metabolic pathways have been shown to differ between T cells of healthy individuals and patients with SLE, and between healthy and lupus-prone animals. Mitochondrial glucose oxidation can be increased[72] and mitochondria have been shown to be hyperpolarized in chronically activated T cells in SLE[73,74]. Persistent mitochondrial hyperpolarization leads to production of reactive oxygen species (ROS), which can sensitize T cells to necrosis, leading to the release of self-antigens and perpetuation of the autoimmune response[75]. The *Sle1c* locus conferred chronic $CD4^+$ T cell activation in the NZB mouse model of lupus[76]. This locus can be further divided, and the *Sle1c2* susceptibility locus contains only two genes, one of which, *Esrrg*, encodes oestrogen-related receptor γ (ERRγ), a nuclear receptor that regulates oxidative phosphorylation and mitochondrial function. Studies by Perry *et al.* in $CD4^+$ T cells from mice expressing the *Sle1c2* locus showed decreased mitochondrial mass and chronic mitochondrial hyperpolarization compared with wild-type $CD4^+$ T cells[77]. Interestingly, B6.*Sle1c2* $CD4^+$ T cells produced more IFNγ than controls. Increased proliferation and activation of B6.*Sle1c2* $CD4^+$ T cells could be attributable to decreased expression of ERRγ — in breast cancer cells, a decrease in levels of ERRγ led the cells to undergo aerobic glycolysis and expend ATP[78]. Although Perry *et al.*[77] did not demonstrate that decreased *Essrg* expression in *Sle1c2* $CD4^+$ T cells, or the effects of this decrease on mitochondrial function, were directly responsible for increased T_H1 skewing, studies have shown that increased glycolysis due to overexpression of GLUT1 in $CD4^+$ T cells increases IFNγ production[16]. Most importantly, the studies in B6.*Sle1c2* mice further confirm a role for mitochondrial metabolism in rheumatologic diseases and suggest that altered T cell metabolism is, in part, genetically programmed.

In addition to changes in glucose metabolism, $CD4^+$ T cells from patients with SLE also display defects in lipid metabolism. T cells from these patients show increased levels of glycosphingolipids and cholesterol, as well as increased expression of the nuclear receptor oxysterols receptor LXRβ (also known as liver X receptor β), which has a role in cellular lipid metabolism and trafficking[79,80]. Treatment of $CD4^+$ T cells from patients with SLE with an LXR antagonist led to decreased glycosphingolipid production, and blockade of glycosphingolipid biosynthesis in these cells restored normal T cell function[50].

Whole-body metabolism can also be affected in SLE, which could influence autoimmunity. Although the underlying mechanisms are poorly understood, patients with SLE had significantly elevated fasting levels of insulin, indicating a predilection for insulin resistance and metabolic disease[81]. This phenomenon was recapitulated in a mouse model of lupus whereby B6.*Sle1.Sle2.Sle3* mice spontaneously developed glucose intolerance without being fed a high-fat diet[82]. Whereas immune dysfunction might contribute directly to the sequelae of metabolic syndrome, such as atherosclerosis[83], altered metabolic hormones and lipids can also modulate immunity, promoting B cell dysfunction[82] and effector T cell differentiation and function[84–86].

Rheumatoid arthritis. Chronic stimulation and the synovial microenvironment alters T cell metabolism in RA. T cells of patients with RA have reduced expression of 6-phosphofructo 2-kinase/fructose-2, 6-bisphosphatase 3 (PFKFB3)[87]. This enzyme is a key regulator of fructose-2, 6-bisphospate, the allosteric activator of phosphofructokinase, and lower PFKFB3 will lower glycolysis while increasing flux to the pentose phosphate pathway and generation of NADPH[7,87]. Elevated NADPH can neutralize ROS, which, although damaging at high concentrations, are otherwise essential to promote T cell activation[88]. Indeed, restoration of T cell ROS could suppress synovial inflammation[89]. In addition to direct changes in T cells, the hypoxic environment in the RA synovium[90] creates a situation similar to the chronic mitochondrial hyperpolarization seen in SLE. The formation of

Box 1 | **Key principles in immunometabolism pharmacology**

Specificity
A critical goal in targeting any pathway is specificity for a population of cells that drives the disease phenotype. Because metabolic pathways are, in principle, shared between all cells, target specificity is a concern when developing new therapies. However, despite potentially shared expression of enzymes, specificity arises from the requirements of immune cells to maintain high metabolic fluxes through specific pathways to elicit specific functions.

Redundancy
Typically, multiple isoforms of each enzyme or multiple transporters for each nutrient exist. Only specific cell populations rely on a given enzyme isoform or transporter, so inhibition of these proteins will affect only that particular population of cells.

Plasticity
Metabolic pathways can adapt to shifts in nutrient availability. Thus, blockade of a specific pathway can simply elicit plasticity and many cells can adjust to bypass the block or to utilize a different pathway. However, these changes in the cellular metabolic programme can modify the function of immune cells. A shift in pathways that might be insufficient to induce apoptosis or block proliferation might nevertheless shift the fate of a T cell or macrophage to reduce or modify inflammatory function.

Partial inhibition
Because metabolic pathways are limited by conservation of mass and, unlike kinase signalling cascades, do not generally amplify, a partial inhibition can lead to a large functional effect.

Durability of response
Concerns of adverse effects will be reduced if the fate of immune cells is shifted so as to elicit durable responses to time-limited or episodic treatment.

the synovial pannus restricts the availability of oxygen to infiltrating immune cells, which might contribute to altered glucose and mitochondrial metabolism[90].

Osteoarthritis. Altered metabolism contributes to OA but the underlying mechanisms are less firmly established than in SLE or RA. Nevertheless, increased glucose uptake, as determined by ^{18}F-fluorodeoxyglucose PET imaging, correlated with OA progression[91]. The hypoxic environment of the OA synovium might promote osteoclast differentiation and function[9]. Furthermore, metabolic syndrome can exacerbate OA[92], and advanced glycation end products (AGEs) can activate the AGE-specific receptor (RAGE) to impair osteoblast growth and function and promote receptor activator of NF-κB ligand (RANKL, also known as TNF ligand superfamily member 11) and osteoclastogenesis[92,93]. Indeed, chondrocyte-synthesized RANKL might promote bone destruction in OA[94]. The role for mitochondria in osteoclast differentiation was established by genetic deletion of a component of electron transport complex I, Ndufs4, in mice. Deletion of Ndufs4 led to greater differentiation of precursor cells into macrophages rather than osteoclasts[95], supporting a model in which mitochondrial oxidative metabolism promotes osteoclastogenesis. This balance is complicated, with oxidative metabolism seemingly important for osteoclast differentiation and glycolysis seemingly important for bone resorption[96].

Targeting immunometabolism

Rationale for targeting immunometabolism in rheumatologic diseases. Given the metabolic changes associated with immune cell activation and function, as well as the altered metabolism of T cells, macrophages and dendritic cells in rheumatologic diseases, a key question is to what extent is it possible to target metabolism with new therapies? The observation of aerobic glycolysis (the Warburg effect) in cancer cells has led to cell metabolism being considered an attractive potential target for cancer treatment for a number of years[97]. However, the effects of strategies directly inhibiting metabolic pathways have been disappointing or generally modest[97]. One very important difference between successful cancer therapies and successful therapies to control inflammatory diseases is that cancer cells must be fully eliminated, whereas simply halting effector function would be sufficient in immunologic diseases. When targeting immunometabolism in autoimmunity, therefore, blocking a metabolic pathway to the extent that apoptosis is induced is not necessary[98]. Rather, it is essential only to impair a pathway sufficiently so as to alter specific cell functions. A variety of pathways could, in principle, be targeted to modulate an immune response. Effector T cells, for example, require high rates of glycolysis and amino acid uptake, whereas T_{reg} cells are less dependent on or can even be independent of these pathways[55–57]. Therefore, it is reasonable to hypothesize that inhibition or modulation of glycolytic pathways could shift the balance of effector and regulatory T cell subsets to provide a favourable outcome in autoimmune disorders. Each of these pathways has multiple metabolic steps and specific enzymes or nutrient transporters amenable to pharmacologic intervention.

Principles of targeting immunometabolism in rheumatologic diseases. Several key principles will dictate approaches to pharmacologic modulation of immunometabolism in rheumatologic diseases (BOX 1). For example, unlike kinase signalling pathways, metabolic pathways are not generally amplificatory and weak inhibitors might be most useful. With kinases, the potential for exponential expansion of signalling cascades typically makes it essential to inhibit the vast majority of kinase activity to elicit a functional effect. Metabolic pathways, by contrast, are limited by the levels of metabolites and conservation of mass. Thus, modest inhibition of a kinase might achieve little, but modest inhibition of a metabolic pathway could have a strong effect. This paradigm is evident in the action of metformin, a weak inhibitor of mitochondrial electron transport complex I[99] that can nonetheless leads to multiple effects that modify cell function and survival, including reducing T_H17 cells and osteoclasts in a model of RA[100] and promoting T_{reg} cell differentiation[46,100]. It stands to reason that this treatment strategy would also be beneficial in other autoimmune disorders characterized by effector T cell dysregulation, such as SLE. Additionally, specificity of a therapeutic approach targeting metabolic pathways can arise not only from restricted expression of the target, but from the dependence of specific cell populations on specific metabolic pathways. Ideally, a pharmacologic target would be selectively expressed only in the target cell type. However, an equivalent outcome can be achieved if the drug target is only essential in a specific population of cells. This seems to be the case for many potential targets in immunometabolism. Such a strategy could be employed by inhibiting HIF-1α to block the development of T_H17 cells and promote T_{reg} cell differentiation in RA and OA. HIF-1α is specifically required

for glycolysis in T_H17 cells, and does not play a part in other T cell subsets. Thus, although fundamental metabolic pathways might be shared, the selective reliance of immune cell subsets or populations on specific metabolic programmes renders those cell populations susceptible to inhibition.

Several strategies might be used to modulate immunometabolism in rheumatologic diseases. In addition to targeting key metabolic regulatory signalling pathways, such as the mTOR pathway[48,49], or direct inhibition of metabolic events, such as nutrient uptake or enzyme function, metabolic pathways could be modulated at bifurcation points in order to shift metabolic flux from one pathway to another. Pyruvate metabolism might provide such a target. Two of the major fates of pyruvate are conversion to lactate by lactate dehydrogenase (LDH) or uptake into mitochondria to generate acetyl-CoA for oxidation by pyruvate dehydrogenase (PDH). Inflammatory effector T cells favour pyruvate conversion to lactate, whereas T_{reg} cells favour pyruvate oxidation[19]. The flux of pyruvate towards lactate or acetyl-CoA can be regulated by PDH kinase (PDHK) phosphorylation and the inhibition of PDH. Thus, effector T cells utilize PDHK to maintain LDH-mediated conversion of pyruvate to lactate. Inhibition of PDHK relieves PDH inhibition to promote pyruvate conversion to acetyl-CoA and impairs effector T cell function while promoting T_{reg} cell differentiation. This strategy has shown promise in relieving inflammation and promoting T_{reg} cells in models of disease including collagen-induced arthritis[101], asthma[102], alloreactivity[103] and experimental autoimmune encephalitis (EAE)[19].

Immunometabolic therapeutic targets

There are many potential targets from which to choose to modulate autoimmunity and improve rheumatologic disease outcomes. Some metabolic processes are already targeted by standard of care treatments for these diseases. Methotrexate, for instance, has many modes of action, including potential inhibition of Janus kinase (JAK)–STAT signalling[104]. Inhibition of one-carbon metabolism (a network of pathways involved in amino acid metabolism and nucleotide synthesis) by methotrexate might also have important inhibitory functions on cell growth, redox balance and epigenetics[105]. Other key areas could also provide focal points for new drug development (FIG. 2); indeed, several examples now exist in which pharmacologic targeting of metabolism has had protective effects against immune-mediated diseases. In an important proof-of-principle study, inhibition of T cell metabolic pathways protected lupus-prone mice from disease: Yin *et al.* showed that treatment with the non-metabolizable glucose analogue 2-deoxy-D-glucose (2-DG) plus metformin reversed cytokine and autoantibody production in an animal model of lupus[106]. Furthermore, *in vitro* production of IFNγ by T cells from patients with SLE was normalized by metformin treatment. The combination of 2-DG and metformin would suppress both glycolysis and mitochondrial metabolism. The extent to which such dual metabolic inhibition might be broadly necessary in the treatment of rheumatologic diseases is unclear, but the metabolic plasticity of T cells might require this approach.

Beyond combinations of 2-DG and metformin, targeting amino acid metabolism could prove a promising approach. One potential therapeutic strategy is inhibition of glutamine uptake and metabolism. Glutamine is a non-essential amino acid that is used at high rates to support anabolic metabolism and its uptake is rapidly increased during T cell activation via the transporter SLC1A5 (REFS 56,107). Importantly, SLC1A5 deficiency attenuates T_H1 and T_H17 responses and prevents the onset of EAE in experimental mouse models[56]. The amino acid transporter SLC7A5 is also essential for T cell activation[57] by supporting amino acid uptake essential for mTORC1 activity. Given the wide role of amino acids in anabolic metabolism and intracellular signalling, mechanisms that regulate these pathways are promising targets for modulation of immune cell function in inflammatory diseases. Strategies to suppress glycolysis, mitochondrial metabolism and amino acid metabolism could have far-reaching applications beyond autoimmunity. A 2015 study demonstrated that the combination of 2-DG and metformin, with the addition of an inhibitor of glutamine metabolism, reduced rejection of skin allografts or heart transplants in mice whereas the individual treatments had minimal effects[108].

Regulation of ROS is also critical for immunological function[88], and mitochondrial ROS production could be a target. Indeed, the F_1F_0-ATPase inhibitor Bz-423 (REF. 109) does not block ATP production but rather leads to increased ROS and can protect against lupus and graft-versus-host disease in animal

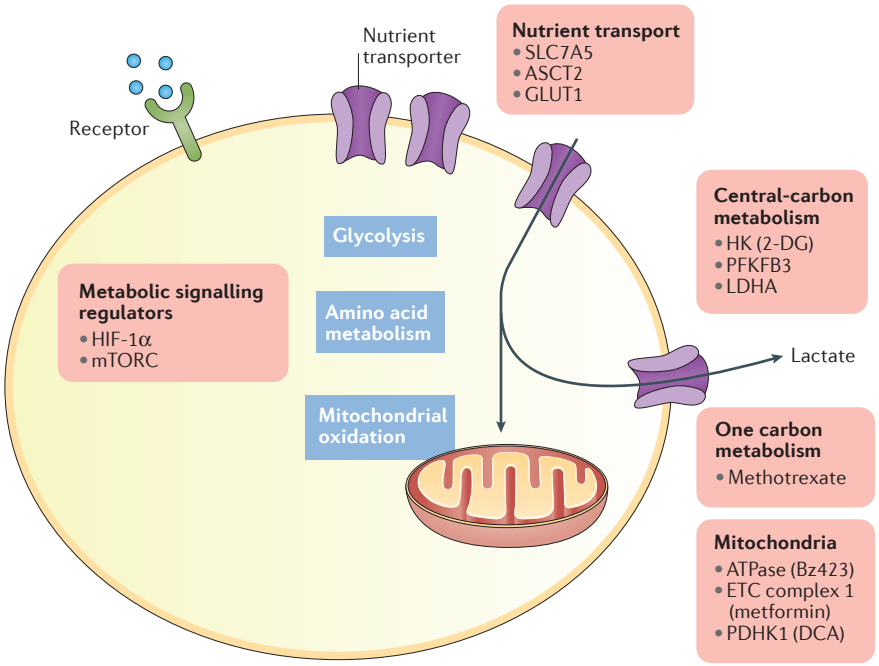

Figure 2 | **Metabolic processes to target in the treatment of rheumatologic diseases.** Metabolic areas and key current or potential targets for drugs to modify immunometabolism and shift immune cell subsets and fate are indicated. 2-DG, 2-deoxy-d-glucose; ASCT2, solute carrier family 1 member 5; DCA, dichloroacetate; ETC, electron transport chain; GLUT1, glucose transporter 1; HIF-1α, hypoxia-inducible factor 1α; HK, hexokinase; LDHA, lactate dehydrogenase A; mTORC, mechanistic target of rapamycin complex; PDHK1, pyruvate dehydrogenase kinase 1; PFKFB3. 6-Phosphofructo 2-kinase/fructose-2,6-bisphosphatase 3.

models, in part by inducing lymphocyte apoptosis[110,111]. PDHK1 can also regulate mitochondrial ROS via regulation of pyruvate flux into the TCA cycle. Indeed, inhibition of PDHK1 led to increased ROS that promoted T_{reg} cells and could protect from EAE[19]. In addition, the mitochondrial ROS scavenger MitoQ reduced mitochondrial anti-viral signalling (MAVS) activation and attenuated IFNγ production[32,112].

A number of other metabolic events have promise as targets in rheumatologic diseases. Given the role of hypoxia in RA and OA, targeting the stability of HIF-1α or HIF-2α and the hypoxic response might offer protection from multiple aspects of joint inflammation[113]. Similarly, modulators of glycolysis, such as PFKFB3 (REF. 114) or LDH[115], can suppress T cell activation or regulate IFNγ production. With these approaches, direct inhibition of a central carbon glucose metabolism pathway raises concerns of broad toxicity. However, in the studies discussed above the effects *in vivo* were surprisingly modest. This outcome is probably due to the partial inhibitory effect of each of these strategies and the selective dependence on those pathways of metabolically active inflammatory cells.

Challenges and future directions

Immunometabolism offers the opportunity to selectively target specific immune cell subsets by modifying the metabolic pathways essential for their function. This concept represents a paradigm shift away from targeting specific signalling pathways that might be active in a wide range of cells. However, a concern is that although only selected cells might require high fluxes through specific metabolic pathways, the extent to which other cell types might also activate and periodically rely on those same pathways remains unclear. Adverse effects of putative metabolic therapies are, therefore, critical challenges. This is particularly true for chronic diseases, which can require long-term treatment. Proliferative or metabolic tissues, such as the gut, liver, muscle and β cells, could be especially sensitive.

Despite these concerns, metabolic pathways are already being targeted, including by standard-of-care therapies, and some metabolic therapies are already standard of care. Other therapies certainly have metabolic implications that might contribute to their mechanisms of action. Methotrexate, for example, inhibits one-carbon metabolism yet is standard-of-care treatment for RA. Also, metabolic changes following inhibition of mTOR signalling certainly contribute to immune suppression[48]. A potential benefit of targeting immunometabolism to modulate immunity is that the selective use of pathways by effector or regulatory T cells or macrophages may enable short-term treatments to shift immune cell populations and provide durable protection from inflammation and disease. Thus, a short therapy period could provide benefit and reduce the potential for adverse effects. The immunometabolism field is rapidly evolving and our increasing knowledge of the metabolic pathways that promote effector and regulatory immune cell differentiation or the generation of osteoclasts might now provide rational strategies to exploit the metabolic requirements of each subset.

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All authors researched the data for the article, provided a substantial contribution to discussions of the content and contributed to writing the article and to review and/or editing of the manuscript before submission.

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CORRIGENDUM

The emerging safety profile of JAK inhibitors in rheumatic disease

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In 'Gastrointestinal perforation' of the 'Adverse effects of JAK inhibitors' section, in the sentence "In patients with RA receiving baricitinib, two cases of gastrointestinal perforations were reported (an incidence of 5 cases per 1,000 patient-years in the development program)" the incidence should have been 0.5 cases per 1,000 patient-years. This error has been corrected in the online version of the article.