Autoinflammation and autoimmunity across rheumatic and musculoskeletal diseases

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Abstract | Most rheumatic and musculoskeletal diseases (RMDs) can be placed along a spectrum of disorders, with autoinflammatory diseases (including monogenic systemic autoinflammatory diseases) and autoimmune diseases (such as systemic lupus erythematosus and antiphospholipid syndrome) representing the two ends of this spectrum. However, although most autoinflammatory diseases are characterized by the activation of innate immunity and inflammasomes and classical autoimmunity typically involves adaptive immune responses, there is some overlap in the features of autoimmunity and autoinflammation in RMDs. Indeed, some 'mixed-pattern' diseases such as spondyloarthritis and some forms of rheumatoid arthritis can also be delineated. A better understanding of the pathogenic pathways of autoinflammation and autoimmunity in RMDs, as well as the preferential cytokine patterns observed in these diseases, could help us to design targeted treatment strategies.

When discussing rheumatic and musculoskeletal diseases (RMDs), it is not always clear whether the disease is strictly an autoimmune disease or is an autoinflammatory disease with unchecked inflammation but without autoimmunity[1–4]. Therefore, it is important to revisit the classification used to describe RMDs[1–4].

When considering whether a disease is an autoimmune disease versus an autoinflammatory disease, systemic lupus erythematosus (SLE) and monogenic systemic autoinflammatory diseases (SAIDs) can be considered as prototypes of autoimmune and autoinflammatory diseases, respectively[3,4]. Autoimmune diseases are characterized by the loss of immune tolerance, the recognition of self-antigens and the activation of T cells and B cells, followed by the production of specific autoantibodies and the damage of multiple organs owing to a dysregulated adaptive immune response[1,3,5]. Autoinflammatory diseases are not directed by specific antigens, and they harbour systemic chronic inflammation without a break in immune tolerance or the generation of specific autoantibodies[4,6]. External environmental factors such as infections, temperature changes or mechanical stress can also lead to the development of inflammation and provoke flare in certain genetic backgrounds, expanding the definition of autoinflammation[4,6].

RMDs are distributed along a spectrum based on the involvement of autoimmunity and autoinflammation in them (FIG. 1). Monogenic SAIDs are at the autoinflammatory end of the spectrum, and SLE and antiphospholipid syndrome (APS) are at the autoimmune end. Rare monogenic autoimmune diseases such as autoimmune polyendocrine syndrome 1, immune dysregulation, polyendocrinopathy, enteropathy, X-linked and autoimmune lymphoproliferative syndrome will not be discussed in this Review as they are not classical RMDs[7]. Diseases related to autoimmunity that are discussed here include SLE, rheumatoid arthritis (RA), polyarticular juvenile idiopathic arthritis (pJIA), systemic sclerosis (SSc), APS, primary Sjögren syndrome (pSS), idiopathic inflammatory myopathies (IIMs), mixed connective tissue disease and antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV)[3,4,8–10] (FIG. 1). As discussed later, a mechanistic immunological classification of RA has been proposed based on the heterogeneity of disease subtypes[8,9]. In addition to monogenic SAIDs, diseases related to autoinflammation and discussed in this Review include gout, spondyloarthritis (SpA), systemic juvenile idiopathic arthritis (sJIA), oligoarticular juvenile idiopathic arthritis, adult-onset Still disease (AOSD), Behçet disease and Schnitzler syndrome[3,4] (FIG. 1). As described previously, most of these autoimmune and autoinflammatory diseases can also be considered to be 'mixed-pattern' conditions[4]. Indeed, there is no strict divide between autoimmune and autoinflammatory diseases as some RMDs comprise elements of autoimmunity and autoinflammation. In such mixed-pattern RMDs,

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Key points

- Rheumatic and musculoskeletal diseases (RMDs) form a continuum between classical autoimmune and autoinflammatory conditions.
- Classical autoinflammatory and autoimmune diseases are associated with the activation of innate immunity and adaptive immune responses, respectively.
- There are some 'mixed-pattern' disorders that carry the features of both autoimmune and autoinflammatory conditions, and one disorder might have autoimmune and autoinflammatory characteristics at different stages of disease development.
- The autoimmune, autoinflammatory or mixed phenotype of RMDs might help us to develop and administer therapies targeted to specific disease phenotypes.

autoantibody-mediated pathology has been observed alongside activation of the innate immune system, including of Toll-like receptors (TLRs) and of the inflammasome. Moreover, immune cells and mediators characteristic of both autoimmunity and autoinflammation can be involved in these diseases[1,3,5,11] (FIG. 1).

Indeed, in terms of immunity, autoimmune and autoinflammatory conditions can have an innate or adaptive immunological background[2,3] (FIG. 2). Innate immunity delivers non-specific cellular and humoral immune responses and confers the first defensive responses against pathogens. Innate immune responses are usually directed against pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). Several molecular systems, including TLRs, NOD-like receptors (NLRs), the caspase recruitment domain (CARD) receptor family, proteins of the complement system, cytoplasmic DNA-sensing molecules and inflammatory multimolecular complexes such as inflammasomes, have evolved to permit diverse recognition and activation and effector function within innate immunity. Immune cells activated during innate immune responses include macrophages, natural killer cells, neutrophils and mast cells (FIG. 2). In addition, other cell types, such as epithelial and endothelial cells, are also induced to express molecules recognizing DAMPs and PAMPs and are classed as 'innate responders'. Epithelial barriers and their dysfunction, partially through alterations in the microbiome, might also play a crucial role in RMDs. The activation of innate immune responses is primarily characteristic of autoinflammation and the development of autoinflammatory diseases (FIG. 1). Within the cytokine superfamilies, the IL-1 family, TNF superfamily members, IL-6 and the type I interferons are particularly implicated in innate immune responses[1,4,12–14].

Adaptive immunity is teleologically younger than innate immunity and exists only in vertebrates. As it enables an immunological memory to form in response to the first encounter with a pathogen, a prompt immune response can develop after consecutive contacts with the same external stimulus. Adaptive immunity is pathogen-specific and driven by T lymphocytes and B lymphocytes, and long-term defence can develop. Temporal and spatial regulation of such a response, as well as its attenuation, is needed to prevent tissue and organ damage. The sustained activation of adaptive immune responses and immunoregulatory defects can lead to the development of classical autoimmune diseases[1–3,5] (FIG. 1).

During the past decade, multiple efforts have been made to better understand the nature of autoimmunity and autoinflammation[1,4], including those using genome-wide association studies, mRNA sequencing, molecular imaging and the study of tissue-specific antigen and gene expression patterns[1,3,4]. In this Review, we first discuss the key features of diseases that are predominantly autoimmune or predominantly autoinflammatory, before describing the overlap between autoimmunity and autoinflammation in RMDs. We also underscore mechanisms shared by autoimmunity and autoinflammation, such as the involvement of pathogenic pathways that are characteristic of autoinflammation in autoimmune conditions (and vice versa), and we highlight how understanding these shared mechanisms might enable us to enhance the efficacy of therapeutics and realize the potential of personalized medicine in rheumatology.

Major features of autoimmune RMDs

SLE, a prototype of systemic autoimmunity, produces more than 100 autoantibody specificities and manifests in various systemic organs (FIG. 1). SLE is based on robust T cell and B cell activation and the formation of immune complexes, whereas cells and mediators that are characteristic of autoinflammation, such as inflammasome activation and the production of IL-1, do not seem to have a major role in this disease[15]. Nonetheless, innate immunity still has an important role in SLE. Indeed, single-nucleotide polymorphisms associated with SLE include those in the genes encoding TLRs (*TLR7* and *TLR9*), complement receptors (*C3*, *C4* and *C1Q*) and Fc receptors (*FCGR2A* and *FCGR3B*), all of which are components of the innate immune response (TABLE 1). The accumulation of 'cellular debris' in tissues and blood in patients with SLE, including as a result of secondary necrosis and the formation of neutrophil extracellular traps (NETs), leads to a breach in immune tolerance and the formation of immune complexes, which triggers the release of inflammatory mediators and organ damage[15,16]. This cell debris-induced breach in immune tolerance is closely linked to dysfunction in complement receptors and Fc receptors. Indeed, mutations in genes encoding proteins of the complement system and the activation of a type I interferon (that is, IFNα and IFNβ) signature, which is also a feature of an innate immune response, are central features of SLE[14,15,17]. The complement genes responsible for susceptibility to SLE are *C1Q*, *C2* and *C4* (REF.[15]). Partial or complete deficiency in C1, C2 or C4 disrupts early steps of the complement cascade, resulting in inadequate clearance of immune complexes. In addition, the Fc receptors FcγRIIIA and FcγRIIIB have anti-inflammatory activity as they clear immune complexes, and mutations in genes encoding these proteins impair this clearance function. In carriers of single-nucleotide polymorphisms associated with SLE, environmental factors that induce cell death, such as ultraviolet light, are necessary for development of the disease[15,18–20]. In SLE, extracellular DNA triggers an *IFN* gene response associated with the production of IFNα and IFNβ. DNA activates *IFN* genes (for example,

IFNA) via the stimulator of interferon genes (STING)–IRF3 pathway and TLR7 and TLR9 (REFS[15,19]). Eventually, the persistence of an interferon signature contributes to disease progression[15,18,21].

The importance of the type I interferon signature and that of other risk alleles associated with components of the innate immune response has also been described in the predominantly autoimmune diseases SSc, IIMs and pSS. For example, in SSc, the type I interferon signature appears early in disease, before the onset of fibrosis, and correlates with an increase in the expression of B cell-activating factor (*BAFF*) mRNA (the protein product of which promotes B cell activation) and an increase in collagen synthesis[22,23]. In the IIMs polymyositis and dermatomyositis, the expression of type I interferon-regulated genes has also been associated with disease activity[24]. Furthermore, high expression of interferon-induced genes has been observed in the skin of patients with dermatomyositis[25]. In pSS, clinical symptoms, disease activity and B cell activation are also associated with the type I interferon signature[26,27]. Finally, certain subsets of RA presumably show a type I interferon signature that promotes the production of autoantibodies such as anti-citrullinated protein antibody (ACPA), anti-carbamylated protein (anti-CarP) and rheumatoid factor[17,28–30], and RA also carries other autoinflammatory features (see below)[8,31,32].

Features of autoinflammatory RMDs

SAIDs comprise an expanding group of diseases, including monogenic diseases caused by inborn errors (also known as periodic fever syndromes) and adult-onset SAIDs such as AOSD, Schnitzler syndrome and idiopathic recurrent autoimmune pericarditis (IRAP)[33–36].

Monogenic autoinflammatory RMDs. In contrast to autoimmune RMDs, monogenic SAIDs are exclusively autoinflammatory conditions[37] (FIG. 1; TABLE 1). A common feature of these diseases, which include both sporadic and monogenic inherited diseases with an overactive innate immune system, is recurrent febrile episodes in the absence of infectious agents. The best described diseases in this group include familial Mediterranean fever (FMF), periodic fever, aphthosis, pharyngitis and adenitis syndrome, hyper-IgD and periodic fever syndrome (also known as mevalonate kinase deficiency), TNF receptor-associated periodic syndrome (TRAPS), Blau syndrome and cryopyrin-associated periodic syndromes (CAPS). CAPS include three diseases caused by mutations in *NLRP3*, the gene encoding the

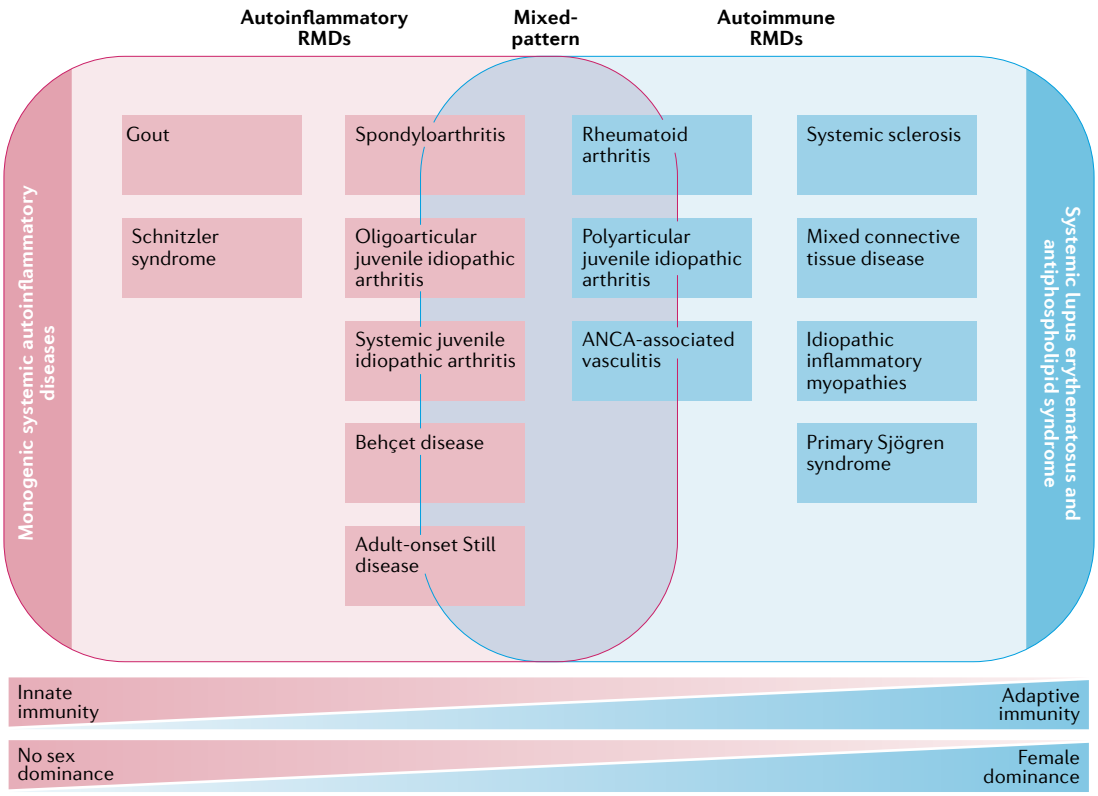

Fig. 1 | **Spectrum of autoinflammatory, mixed-pattern and autoimmune diseases.** Prototypes of a classical autoinflammatory disease are the group of monogenic systemic autoinflammatory diseases known as periodic fever syndromes (pink). Prototypes of classical autoimmune disease are systemic lupus erythematosus and antiphospholipid syndrome (blue). Diseases in the middle of the spectrum might be considered mixed-pattern rheumatic and musculoskeletal diseases (RMDs; mixed colour). Indicated by the spectra at the bottom of the figure, classical autoinflammatory conditions are characterized by a predominance of innate immunity and have no sex dominance. By contrast, classical autoimmune conditions are associated with more prominent adaptive immune responses and female dominance. ANCA, antineutrophil cytoplasmic antibody.

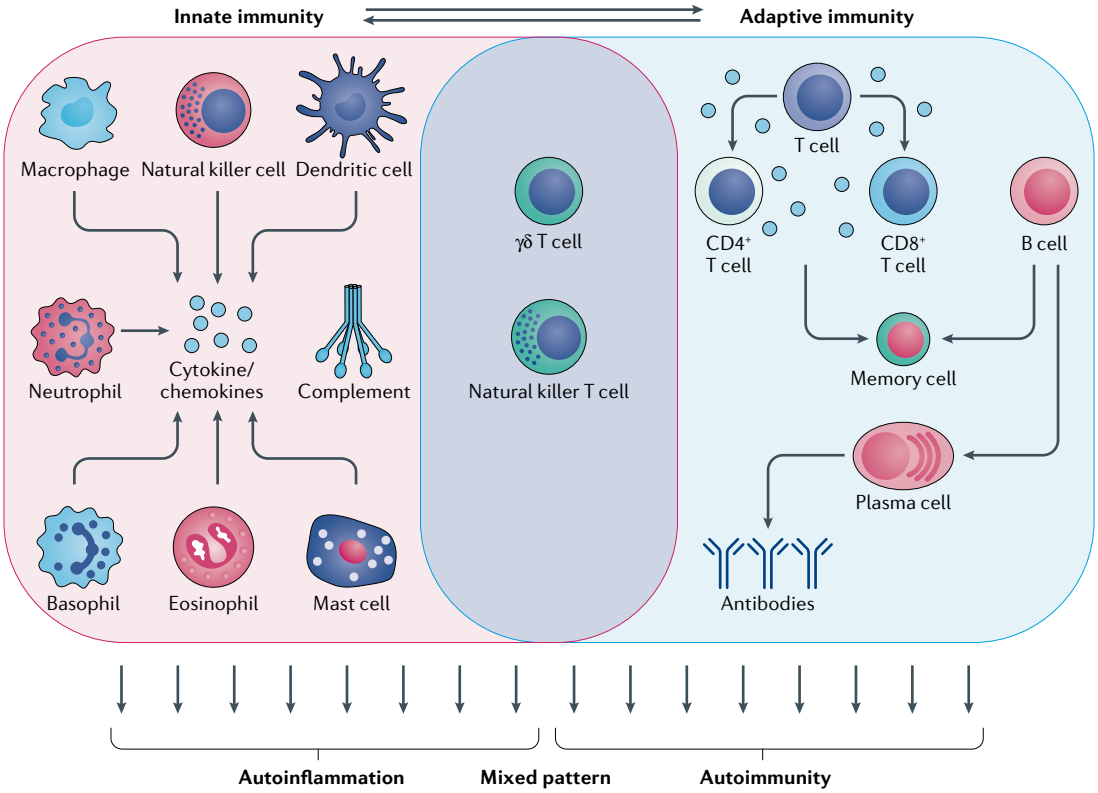

Fig. 2 | **Cellular mediators of autoimmunity and autoinflammation.** Cells of the innate immune system, including macrophages, natural killer cells, dendritic cells, mast cells and different granulocyte subsets, and the complement system promote autoinflammation. Cells of the adaptive immune system, including different T lymphocyte subsets, B cells and plasma cells, as well as T memory cells and B memory cells, are primarily involved in the development of autoimmunity. Natural killer T cells and γδ T cells are at the crossroads of autoinflammation and autoimmunity and promote the development of mixed-pattern immune-mediated inflammatory diseases. Most of the cells involved in the development of autoinflammation and autoimmunity produce cytokines and chemokines (as indicated by the blue circles), whereas plasma cells release antibodies.

NLRP3 protein, namely familial cold autoinflammatory syndrome, Muckle–Wells syndrome and chronic infantile neurologic cutaneous and articular syndrome[38,39]. The clinical features of these monogenic SAIDs have been discussed elsewhere[37–39]. Most of these diseases are caused by inborn errors, although some such as FMF, TRAPS, CAPS, hyper-IgD and periodic fever syndrome, deficiency of adenosine deiminase 2 (ADA2), periodic fever, aphthosis, pharyngitis and adenitis syndrome, and type I interferonopathies can also have adult onset[33,34]. Monogenic SAIDs are mostly associated with mutations in *MEFV*, the gene encoding pyrin, *NLRP3*, or other genes encoding proteins that regulate inflammation, metabolism and body temperature (for example, *NOD2*; also known as *CARD15*)[37,39–41] (TABLE 1). Currently, our understanding of monogenic SAIDs is moving from a gene-centric view towards a systems-based view, and various convergent pathways — such as pyrin and the actin cytoskeleton, protein misfolding and cellular stress, NF-κB dysregulation and interferon activation — have been associated with autoinflammation in SAIDs[42].

Molecular pathways underlying autoinflammation. Activation of the NLRP3 inflammasome and the IL-1β pathway are key events in the pathogenesis of most monogenic SAIDs and polygenic SAIDs (introduced below)[12,43,44]. In the presence of a characteristic genetic mutation, certain external environmental factors (for example, infection, smoking or hormonal factors) can cause uncontrolled activation of the inflammasome, resulting in the development of a cytokine-mediated systemic inflammatory condition[12,43,44]. DAMPs and PAMPs are involved in the initiation of inflammasome activation. Activation of the NLRP3 inflammasome is mediated by the NLR family protein NLRP3 and leads to the activation of caspase 1, which cleaves the cytokine precursors pro-IL-1β and pro-IL-18 to produce the biologically active forms of IL-1β and IL-18, respectively[12,40,41,43]. In response to increased production of IL-1β and IL-18, the endogenous cytokine antagonists IL-1 receptor antagonist (IL-1Ra) and IL-18 binding protein (IL-18bp) restore the balance of these cytokines in the body[12,40,41,43]. Loss of function mutation in genes encoding cytokine antagonists also leads to increased activation of IL-1α and IL-1β (REFS[40,41]).

Activation of NF-κB signalling contributes to the development of certain autoinflammatory diseases, and NOD2, a NLR family protein in addition to NLRP3 that recognizes bacterial dipeptides, is an important regulator of NF-κB signalling. *NOD2* mutation has a role

in the pathogenesis of Blau syndrome and in Crohn's disease[40].

Monogenic SAIDs associated with IL-1β family activation include FMF, familial cold autoinflammatory syndrome, chronic infantile neurologic cutaneous and articular syndrome, hyper-IgD and periodic fever syndrome, Muckle–Wells syndrome and pyogenic arthritis, pyoderma gangrenosum and acne[40,41]. The different gene mutations present in each disease result in activation of the NLRP3 inflammasome and uncontrolled secretion of IL-1β (REFS[40,41]). In addition to IL-1β and IL-18, TNF is also involved in the pathogenesis of some monogenic autoinflammatory disorders[40,41]. Other pathogenetic mechanisms that affect innate immunity and have been implicated in the pathogenesis of SIADs include NF-κB activation, endoplasmic reticulum stress, mutations in genes encoding endogenous cytokine antagonists, dysregulation of actin filament formation (in actinopathies), enhanced expression of *IFN* (in interferonopathies) or a reduction in the enzymatic activity of ADA2 (REFS[33,34]). TRAPS, which is one of the most prevalent monogenic SAIDs, is associated with heterozygous variants in *TNFRSF1A*, the gene encoding TNF receptor 1 (REFS[33,45,46]). Possible pathogenic mechanisms of TRAPS include enhanced NF-κB and NLRP3 activation through increased endoplasmic reticulum stress, defective autophagy or defective receptor shedding leading to TNF-induced cell death and, eventually, autoinflammation[33,45,46].

Polygenic autoinflammatory RMDs. Among polygenic autoinflammatory conditions we will discuss sJIA and gout, two well-known prototypes. sJIA is a typical autoinflammatory disease associated with fever, rash, hepatosplenomegaly and lymphadenopathy, especially in the early, acute phase[47]. Genetic and epigenetic changes are associated with this disease but, although mutations have been described in several genes, unlike in periodic fever syndromes, none of these mutations alone results in sJIA[47]. Gene mutations characteristic of monogenic diseases (for example, mutations in *NLRP3*, *NOD2* and *MEFV*) are not observed in sJIA[47]. sJIA has, rather, been associated with genes encoding pro-inflammatory cytokines (such as *IL1*, *IL1R*, *IL6*, *IL10* and *IL20*) and other mediators of inflammation (such as *IL8* and *MIF*; *MIF* encodes macrophage migration inhibitory factor)[47] (TABLE 1). The proteins encoded by these genes are involved in the innate immune response and, ultimately, create an inflammatory microenvironment; the activation of effector T cells only occurs as a consequence of autoinflammation[3,47]. In the more advanced stage of sJIA, activation of the adaptive immune system and joint tissue destruction can be observed, suggesting that sJIA is associated with the activation of innate and (to a lesser extent) adaptive immunity at different stages of the disease[48,49]. Nonetheless, B cell-mediated autoimmunity is absent in sJIA. Important questions are how and when spurious inflammation in sJIA switches to chronic inflammation[1,49], and whether this switch can be prevented or delayed by early intervention with anti-IL-1 or anti-IL-6 strategies[50].

Autoinflammation is also essential in the development of gout and the central event of gouty inflammation is the activation of white blood cells by monosodium urate (MSU) crystals[12,51,52]. Cell membrane damage by activated leukocytes and their mediators results in the activation of pattern recognition receptors, inducing a response against cellular debris to try to minimize the damage. MSU crystals act as DAMPs and are phagocytosed through TLR2 and TLR4 to form a phagolysosome. Phagolysosome formation is followed by activation of the NLRP3 inflammasome, which leads to the activation of caspase 1 and to the release of IL-1β and IL-18 (REFS[12,51,52]). The production and release of the pro-inflammatory cytokines IL-1, IL-6 and TNF from cells of the innate immune system, independent of inflammasome activation, initiate an inflammatory cascade in which additional mediators of inflammation, such as matrix metalloproteinases, prostaglandins, leukotrienes and reactive oxygen species, also play a role[12,51].

Although monogenic SAIDs, sJIA and gout are the prototypes of autoinflammatory RMDs, AOSD, Behçet disease, IRAP, synovitis, acne, pustulosis, hyperostosis, osteitis syndrome and Schnitzler syndrome can also be classified as adult-onset SAIDs[33–35,38] (FIG. 1). AOSD is an acquired fever syndrome characterized by well-defined clinical (intermittent fever, typical rash and arthritis) and laboratory (hyperferritinaemia, leucocytosis, neutrophilia and abnormal transaminase levels) features. AOSD has been associated with an increased production of cytokines, including of IL-1, IL-6, IL-18 and TNF[53]. Activation of the NLRP3 inflammasome and pathological IL-1 signalling have also been observed in patients with AOSD[53]. Mutations in *MEFV* and *TNFRSF1A* (the gene encoding TNF receptor 1) have been described in patients with AOSD, linking AOSD to monogenic SAIDs[54] (TABLE 1). Behçet disease is a systemic vasculitis affecting the small vessels, and most commonly manifests as mucosal and genital ulcers and uveitis.

Table 1 | **Genes associated with common autoimmune and autoinflammatory disorders**

Classification	Disease	Associated genes
Autoimmune diseases	Systemic lupus erythematosus	*TLR7*, *TLR9*, *C3*, *C4*, *C1Q*, *FCGR2A*, *FCGR3B*, *IFNA*
	Systemic sclerosis	*IFN* signature genes
	Idiopathic inflammatory myopathy	*IFN* signature genes
Autoinflammatory diseases	Monogenic systemic autoinflammatory diseases	*NLRP3*, *NOD2*, *MEFV*, *TNFRSF1A*, *MVK*, *TNFAIP3*, *ADA2*, *TREX1*, *UBA1*
	Systemic juvenile idiopathic arthritis[a]	*IL1*, *IL1R*, *IL6*, *IL10*, *IL20*, *IL8*, *MIF*
	Adult-onset Still disease[a]	*MEFV*, *TNFRSF1A*, *NLRP3*
	Behçet disease[a]	*MEFV*, *TNFRSF1A*, *NLRP3*, *HLAB51*
Mixed-pattern diseases[a]	Ankylosing spondylitis	*HLAB27*, *ERAP1* (also known as *ARTS1*)
	Rheumatoid arthritis	*HLADRB1*, *PTPN22*, *NLRP3*, *MEFV*, *NOD2*

This table is not comprehensive and shows only the most common diseases and their genetic associations. [a]Diseases that can also be mixed-pattern diseases.

In addition to other cytokines, the NLRP3–IL-1 system is important in the development of Behçet disease, meaning that this is a predominantly autoinflammatory condition that can also have mixed-pattern features (see below)[55–57]. Again, mutations in *MEFV* and *TNFRSF1A* are more common in this disease compared with other autoinflammatory conditions[4]. Schnitzler syndrome is also an acquired fever syndrome and is characterized by chronic urticaria associated with monoclonal gammopathy, recurrent fever, bone pain and arthralgia. It is considered to be a neutrophil dermatosis with notable involvement of neutrophils, cells that are involved in innate immunity[58]. Hereditary factors are unlikely to play a role in the pathogenesis of this disease based on its late onset in patients[33,36,59,60].

Mixed-pattern RMDs

Diseases with features of both autoinflammatory and autoimmune RMDs include SpA and some forms of RA. These disorders have also been termed mixed-pattern RMDs[4] (FIG. 1).

As well as ankylosing spondylitis (AS) and psoriatic arthritis (PsA), forms of SpA include enteropathic arthritis (also known as inflammatory bowel disease-associated arthritis), reactive arthritis and undifferentiated SpA[61,62]. In contrast to classical autoimmune diseases, SpA is associated with HLA-B but not with HLA-DR, which is characteristic of polygenic autoimmune diseases[61,63–65]. Moreover, unlike other autoimmune diseases, there is no female dominance in SpA. Furthermore, SpA has been associated with autoantibodies; some patients with AS and PsA have autoantibodies to mutated citrullinated vimentin, CarP, sclerostin, heat shock proteins or β_2-microglobulin[61,63–65]. CD74 is the invariable γ-chain of MHC class II, and anti-CD74 antibodies are considered to be specific for SpA in European but not Asian cohorts[65]. Among cytokines, in addition to TNF, IL-17 and IL-23 seem to have a predominant role in mixed-pattern RMDs[61,66]. Associations of SpA with mutations in *ERAP1* (also known as *ARTS1*, encoding endoplasmic reticulum aminopeptidase 1) and with MHC class I suggest that T cells interact with cytokine pathways, including the IL-23–IL-17 axis but not the IL-1 pathway, in patients with this disease[56,57,67] (TABLE 1). In terms of autoinflammation, NLRP3 and caspase 1 are upregulated in AS, suggesting that autoinflammation is involved in the pathogenesis of this disease[68]. In short, features of both autoimmunity (such as autoantibodies) and autoinflammation (such as gender balance and natural immune responses to microbial pathogens) have been identified in SpA[61].

RA generally has autoimmune features in the early phase of the disease but has a macrophage and fibroblast-dominated pathogenesis in the chronic phase. Thus, RA is an example of a condition in which the phase of the disease relates to its autoimmune or autoinflammatory nature[4,10,30,69]. Five patients with seropositive RA had HLA-DRB1*01 and/or HLA-DRB1*04 shared epitopes as well as mutations in *NLRP3*, *MEFV* or *NOD2* (REF.[9]) (TABLE 1). These patients showed features of autoinflammation and responded to colchicine[9]. Based on the findings of this study, the authors proposed an immunology-based reclassification of RA that includes classical seropositive autoimmune RA, autoinflammatory seronegative forms of RA and mixed forms of RA that are seronegative[8,9]. This proposed reclassification reflects the commonly accepted idea that RA is a syndrome based on different pathophysiologic events rather than a single disease.

Juvenile idiopathic arthritis can also be a mixed-pattern disease with both autoinflammatory and autoimmune features. For example, pJIA shares many of the features of adult RA described above[47,70]. Also, although sJIA is largely considered to be a SAID dominated by innate immunity-driven inflammation, in later stages it can progress towards an adaptive immunity-dependent arthritis[47–49].

Among diseases primarily considered to be autoinflammatory, AOSD and Behçet disease have also been associated with adaptive immunity and T cell responses and thus can also be considered mixed-pattern conditions[4,56,57]. AOSD can be systemic with predominantly autoinflammatory features or have a chronic articular pattern resembling classical RA, which could have relevance for therapy. For example, different phenotypes of AOSD respond to different biologics[4,71]. Moreover, genetic analysis has confirmed that sJIA and AOSD might form a continuum of a single disease. Specifically, sJIA and AOSD can share common genes, and the differentiation between these two diseases is mainly based on the age of onset[35]. Behçet disease, a primarily autoinflammatory condition, is also associated with the MHC class I molecule HLA-B51, notable T cell responses and the production of IL-23 and IL-17 (REFS[56,57]), highlighting that it also has features of autoimmune conditions.

Finally, among monogenic SAIDs, haploinsufficiency of A20 — which is caused by mutations in *TNFAIP3*, the gene encoding the NF-κB regulatory protein A20 (REFS[33,72]) — is a good example of a condition with autoimmune and autoinflammatory features that result from the same pathogenetic pathways. This disease carries characteristics of RA, gout, Behçet disease, AOSD, SLE, periodic fever, aphthosis, pharyngitis and adenitis syndrome, as well as skin, ocular and gastrointestinal symptoms. Therefore, diagnosis and differential diagnosis of haploinsufficiency of A20 is difficult[72].

In conclusion, mixed-pattern RMDs carry both classical autoimmune and autoinflammatory features and are often associated with non-rheumatic conditions[1,3,4,8].

Innate immunity in autoimmune RMDs

Having discussed the main features of autoimmune, autoinflammatory and mixed-pattern RMDs, it is important to consider the innate immune mechanisms that most commonly occur in both autoinflammatory and autoimmune diseases.

We have already discussed activation of the NLRP inflammasome and the consequent production of IL-1β and IL-18 in autoinflammation[12,44]. However, these features have also been demonstrated in autoimmune and mixed-pattern conditions. NLRP3 activation and the consequent production of cytokines, as well as relevant genetic polymorphisms (for example, in *NLRP3* and

NOD2), have been associated with RA[30,73–76], SpA[77,78], pJIA and oligoarticular juvenile idiopathic arthritis[70]. NLRP3 is also activated, with inflammasome activation leading to tissue injury, in autoimmune RMDs such as RA[79,80], SLE[76,81,82], SSc[83,84], pSS[85] and IIMs[86]. TLR-dependent pathways and abnormal TLR signalling are also characteristic for SLE, RA and other autoimmune RMDs[82].

Type I interferon is upregulated in genetically based interferonopathies, which are not always linked to autoimmunity. STING is a DNA sensor, and a mutation in the gene encoding this protein can lead to the induction of genes involved in IFNα and INFβ-mediated responses and thus, indirectly, the synthesis of numerous pro-inflammatory cytokines[14,40,87]. Rare examples of these interferonopathies also include STING-associated vasculopathy with onset in infancy as well as Aicardi–Goutiéres syndrome[14,40,87]. As discussed above, type I interferon signatures play a key role in autoimmune diseases such as SLE and can also be involved in RA and SSc[87].

NETs are web-like structures of decondensed chromatin, histones and antimicrobial peptides that are involved in the defence against pathogens[58,88–90] and, primarily, have a role in autoinflammatory conditions such as gout[91,92] or Schnitzler syndrome[58]. In gout, the formation of NETs might also be a counter-regulatory mechanism aimed at resolving inflammation[91,92]. Specifically, NETs can stop gout episodes by inducing neutrophil death, encapsulating MSU crystals and inactivating cytokines[91,92]. However, neutrophil activation and NET formation contribute to autoimmune-mediated inflammation in SLE[90,93], RA[90,92] and AAV[90,92].

Prolonged innate immunity-based inflammation can induce adaptive immune responses, as described above for sJIA[48]. However, this phenomenon can also be observed in other RMDs. In monogenic SAIDs and other autoinflammatory diseases, an acute 'hyper-inflammatory state' leading to the resolution of inflammation within days and a prolonged 'autonomous inflammatory state' have been proposed to occur[49,94]. In the latter state, prolonged IL-1β and IL-18 production, in part in synergy with IL-6 and IL-23 activation, can promote T cell differentiation, the induction of T helper 17 cells (T_H17 cells) and the production of IL-17 (REFS[49,95]). Moreover, IL-18 can induce adaptive T_H1 cells and B cells[49]. Thus, innate immunity is involved in some autoimmune RMDs. Finally, a sustained innate immune response can induce trained immunity in autoimmune RMDs, which can contribute to the activation of adaptive immune pathways[49,96].

Comorbidities associated with RMDs

Comorbidities are associated with many RMDs and determine their outcome. The most relevant comorbidities are cardiopulmonary disease (including cardiovascular disease, IRAP and interstitial lung disease (ILD)), osteoporotic fractures, neuropsychiatric manifestations, diabetes mellitus and malignancies[97,98].

The inflammatory condition accelerated atherosclerosis and the consequent cardiovascular disease can carry both autoimmune and autoinflammatory features[99–101]. The autoantibodies ACPA[102,103] and anti-carP[104] might be involved in the development of atherosclerosis in RA. Citrullinated proteins have been detected in the atherosclerotic plaque, suggesting a possible target for ACPA in RA[103]. With respect to autoinflammation, in one large study *NLRP3* gene polymorphisms were not associated with cardiovascular disease in RA[105], whereas in another cohort the presence of the *NLRP3*^{Q705K} minor allele doubled the risk of stroke (also known as transient ischaemic attack) but did not increase the risk of myocardial infarction in RA[106]. In patients without rheumatic disease, NLRP3 and caspase 1 transcripts are abundantly expressed in atherosclerotic plaques[107]. Polymorphisms in CARD-containing protein 8 were not associated with any type of cardiovascular event in RA[106]. With respect to pro-inflammatory cytokines, inflammatory atherosclerosis associated with RMDs has been characterized by the increased production of TNF and IL-6 (REFS[99,100]). In addition, both IL-1 and IL-18 are abundantly produced in the atherosclerotic plaques[107,108], and IL-18 is a predictor of mortality in patients with cardiovascular disease[109]. In patients with SLE, IL-18 production has also been associated with kidney damage and cardiovascular disease[82].

The comorbidity IRAP should also be considered when monitoring and treating RMDs. Recurrent pericarditis can occur in viral infections but can also be associated with various autoimmune RMDs (for example, SLE, SSc, IIMs, pSS and RA) and autoinflammatory RMDs (for example, FMF, TRAPS and Behçet disease)[110,111]. IRAP can carry some autoimmune features as it has been linked to the production of anti-heart and anti-intercalated disk autoantibodies, as well as to autoreactive T cells[110]. However, IRAP has also been associated with notable NLRP3 activation, and cases resistant to NSAIDs, corticosteroids and/or colchicine might respond well to the inhibition of IL-1 (REFS[110,111]). Based on these observations, IRAP can also be considered an autoinflammatory disease[110–112].

ILD is mostly associated with autoimmune conditions such as SSc or IIMs, and the presence of specific autoantibodies, such as anti-Scl70, anti-PLββ-7 and anti-PL-12, correlates with an increased risk of developing ILD in these diseases[113,114]. By contrast, there is limited information on the possible involvement of autoinflammation in ILD. One study investigated the role of NLRP3 inflammasomes in patients with idiopathic pulmonary fibrosis and in patients with RA and usual interstitial pneumonia. IL-1β and IL-18 levels were elevated in bronchoalveolar lavage fluid and bronchoalveolar lavage fluid macrophage cultures from patients with RA and usual interstitial pneumonia compared with healthy individuals[115]. However, the role of autoinflammation in ILD has not been confirmed.

A great number of autoimmune (for example, SLE), autoinflammatory (for example, TRAPS and FMF) and mixed-pattern (for example, Behçet disease) diseases also have neuropsychiatric comorbidities. Based on the nature of these manifestations, these comorbidities might not have the same pathogenesis; however, neuroinflammation could be a common link between these disorders[4,57,116,117].

Finally, most RMDs have been associated with generalized bone loss leading to osteoporosis and fragility fractures[68,97,118]. Proinflammatory cytokines, such as TNF, IL-1, IL-6 and IL-17 (REF.[118]), as well as various DAMPs, including purine metabolites and fatty acids, have been implicated in inflammatory bone disorder[68]. Cytokines and DAMPs both stimulate NLRP3 and NLRC4 inflammasomes, and NLRP3-deficient mice are protected from bone loss[68]. Thus, autoinflammation is implicated in osteoporosis that occurs secondary to RMDs.

Treating RMDs across the spectrum

The pathogenesis of autoimmunity and autoinflammation, especially the cytokine networks characteristic of these conditions, might enable effective targeting strategies[43,66,119].

Treating autoinflammatory diseases. Autoinflammation often responds well to recombinant IL-1RA (anakinra), anti-IL-1β antibody (canakinumab) or recombinant IL-1R fusion protein (rilonacept)[119–121]. Canakinumab has been registered for the treatment of CAPS, TRAPS, FMF, AOSD, sJIA and refractory gouty flares[122,123]. In addition, rilonacept[124,125] and anakinra[126] are also effective in treating monogenic SAIDs. Among the less common monogenic SAIDs, recombinant IL-18bp can be administered in NLRC4 inflammasome-associated diseases caused by the overproduction of IL-18 (REF.[41]). In autoinflammatory diseases associated with NF-κB activation, such as TRAPS, IL-1 inhibitors are the first-choice treatment; however, TRAPS also responds well to TNF inhibitor therapy as the TNF receptor activates the NF-κB pathway[41]. With respect to gout, IL-1 inhibitors are effective in treating refractory flares, with most data available for canakinumab[12,127], although rilonacept[128] and anakinra[129,130] are also effective in treating gouty flares. For patients with sJIA, canakinumab[131,132], the anti-IL-6 receptor antibody tocilizumab[133] and anakinra[134] are registered for treatment, and rilonacept[135] is also effective in treating this disease. Canakinumab[136] and anakinra[126,137] are effective in, and registered for, treating patients with AOSD. Rilonacept can be administered off-label to patients with AOSD[137], and TNF and IL-6 inhibitors are also effective in treating patients with AOSD[32,138]. IL-1 inhibitors, such as canakinumab and anakinra, also showed efficacy in treating patients with Behçet disease[139]. All IL-1 inhibitors are also effective in patients with Schnitzler syndrome[36,140].

Treating autoimmune diseases. In autoimmune diseases, T cells, B cells and their cytokines play a notable role in disease pathogenesis, and the B cell inhibitor rituximab can be used off-label for treating most autoimmune diseases, including SLE[141], SSc[142], dermatomyositis[143] and pSS[144]. Belimumab, an anti-BAFF antibody, has been approved for the treatment of SLE[145], and the CTLA4–Ig fusion protein abatacept can also be administered to inhibit T cells in selected cases of SLE[146], SSc[147] and pSS[148]. It is also possible that cytokines that activate T_H17 cells (such as IL-17 and IL-23) and are used to treat RMDs with a mixed innate (neutrophil activation) and adaptive (T cell activation) background (such as AS and PsA) might also effectively treat classical autoimmune diseases. By contrast, cytokine inhibitors such as those that block IL-1 and TNF, which are effective in autoinflammatory diseases and in diseases such as RA with both autoinflammatory and autoimmune features, show limited efficacy in these autoimmune diseases. However, the IL-6 inhibitor tocilizumab gave promising results in SSc[149] and might be tried in the treatment of other autoimmune diseases[150,151].

TNF appears to be an excellent target in many inflammatory diseases, such as RA, AS, PsA and pJIA[66]. However, it might not be the optimal target in classical autoimmune disorders, such as SLE, SSc, AAV or pSS[66].

Treating mixed-pattern diseases. JAK inhibitors have been approved for treating RMDs with a mixed innate and adaptive immune activation, such as RA and SpA, and preliminary data suggest that they show promise for the treatment of patients with SLE, IIM, pSS, type I interferonopathies, sJIA, AOSD, Behçet disease and monogenic SAIDs[152]. Mixed-pattern diseases could also be treated with a combination of therapeutic strategies. For example, haploinsufficiency of A20, AOSD, Behçet disease or sJIA can be treated with TNF, IL-1 or IL-6 inhibitors based on the dominance of autoinflammatory versus autoimmune features in the patient[66,71,72].

Finally, trials to inhibit common molecular mechanisms of autoinflammation and autoimmunity, such as inflammasomes or NETs, have been carried out[89]. Several inflammasome inhibitors that target components of the NLRP3 cascade are under investigation for the treatment of autoinflammatory conditions[12,44,153]. Among currently used anti-rheumatic drugs, antimalarials and JAK inhibitors also inhibit NETs[89]. Some inhibitors of the protein arginine deiminase enzyme involved in protein citrullination might also block NET formation[89].

Conclusions

Autoimmune and autoinflammatory RMDs can be considered to be a spectrum of disorders. Monogenic SAIDs, and SLE and APS, are likely to represent the two ends of this spectrum of RMDs. Autoinflammatory diseases such as gout, sJIA, Behçet disease, AOSD or Schnitzler syndrome are characterized by the activation of innate immunity, whereas classical autoimmune diseases such as SSc, IIM, pSS, mixed connective tissue disease or seropositive RA are associated with adaptive immune responses and the production of autoantibodies. In addition to the fact that both autoinflammatory and autoimmune diseases can carry some features of the other disease type, there are mixed-pattern diseases that include SpA, AAV, pJIA, oligoarticular juvenile idiopathic arthritis and some forms of RA. The involvement of characteristic pathogenic proteins or pathways, such as of PAMPs, DAMPs, pattern recognition receptors, complement or inflammasome activation in autoinflammation, or of type I interferon signatures and the production of autoantibodies in autoimmunity, along with preferential cytokine patterns, might help inform the design of directed treatment strategies.

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Review article

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Immune-cell profiling to guide stratification and treatment of patients with rheumatic diseases

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Abstract

Methods for high-dimensional immune-cell profiling have advanced dramatically in the past decade. Studies of tissue and blood samples from patients with rheumatic diseases have revealed stereotyped features of immune dysregulation in individual diseases and in subsets of patients who share diagnosis of a heterogeneous disease. Translating immunological patterns into clinically implementable, actionable biomarkers has the potential to improve detection and quantification of pathological immune activity and selection of appropriate treatments for autoimmune rheumatic diseases. For example, cytometric features can be used to distinguish the various forms of inflammatory arthritis, stratify subsets of patients with rheumatoid arthritis or subsets of patients with systemic lupus erythematosus and predict treatment responses. Cellular immune profiling also enables the identification of specific features of immune dysregulation in individuals with rare, undiagnosed, inflammatory diseases. Several paths might lead to translation of discoveries from broad immune profiling into clinical tests to interrogate immune activation in people with rheumatic diseases.

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Distinct immune-cell features across rheumatic diseases

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Conclusion

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Key points

- Profiling of immune cells in blood and tissue from patients with rheumatic diseases has helped to define populations of activated immune cells that are characteristically expanded in specific diseases, highlighting both unique and shared features across diseases.
- Immune profiling of patients with SLE has identified specific axes of immune dysregulation, including activation of type I IFN pathways, proliferation of lymphocytes, expression of cytotoxic molecules on T cells and upregulation of myeloid cell- and neutrophil-associated signatures; these features vary across patients and help to delineate subgroups of patients that differ in immune activity.
- Longitudinal evaluation of cellular profiles of patients receiving treatments targeting rheumatic disease helps to associate immunological features with treatment effects and predict response to treatment.
- Incorporation of immune profiling into clinical evaluation of patients with rheumatic diseases might enable improved patient stratification, assessment of disease activity and prediction of treatment response.

Introduction

Individuals who seek rheumatological evaluation for a possible autoimmune rheumatic disease commonly describe that they feel 'inflamed' and often wonder whether their immune system is 'overactive'. A major challenge for the evaluating physician is to determine whether the immune system has become pathologically activated, driving an autoimmune or inflammatory condition, or whether symptoms are caused by non-immune mechanisms. These decisions are impactful, as they might dictate whether immunosuppressive therapies will be used. Yet, currently available laboratory tests provide rheumatologists with a quite limited assessment of an individual's immune status at any given time.

Routine immunological tests include a complete blood count to determine if the major blood-cell lineages (neutrophils, monocytes, lymphocytes) are present at normal levels. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) tests provide measures of systemic inflammation[1]. Total immunoglobulin levels determine whether the immune system has made antibodies at normal levels, and autoantibody tests demonstrate the presence of antibodies that are, for example, specific to double-stranded DNA or antibodies to citrullinated proteins. Tests of serum complement levels evaluate whether the complement cascade has been activated, for example, by antibody–antigen immune complexes[2]. However, this set of widely used tests provides limited insight into the level of activity of the immune system. In contrast to the range of functional tests to assess cardiac, pulmonary, renal and hepatic function, we currently lack functional tests to assess normal versus aberrant activity of the immune system.

Serum protein profiling, bulk RNA sequencing (RNA-seq) of whole blood or specific cell populations and cytometric profiling — which is the focus of this Review — have been used to interrogate the activity of the immune system in a research setting. Over the past 10 years, the advent of high-dimensional cellular-profiling technologies, including spectral flow cytometry, mass cytometry and single-cell RNA-seq (scRNA-seq), have provided a powerful set of tools for analysing the composition and functional states of immune cells in human blood and tissues. Studies using these methods are providing a new set of metrics for assessing normal and aberrant immune profiles, emphasizing abnormalities in the abundance or activation states of immune-cell populations. These metrics have the potential to complement the current clinical evaluation of rheumatic diseases, including evaluation of rheumatoid arthritis (RA) (Box 1).

This Review discusses insights from selected immune-profiling studies that have highlighted specific features of immune-cell dysregulation across rheumatic diseases, across subgroups of patients with the same rheumatic disease or in individuals with very unusual clinical presentations. This discussion covers only a subset of the wide range of immune-profiling studies that have been performed in past years, with an emphasis on studies of RA and systemic lupus erythematosus (SLE) that involve broad cytometric profiling approaches, including approaches with single-cell resolution; studies using serum proteomics or bulk transcriptomics are noted in some cases to provide context or complement interpretation of cellular-profiling studies.

High-dimensional cellular profiling

Cytometric profiling with flow cytometry, mass cytometry or single-cell transcriptomics enables the quantification of the various cell populations and activation states within a complex mixture of cells. Flow cytometry methods have been well established for decades, using antibodies tagged with fluorophores to quantify the expression of cell-surface or intracellular proteins with single-cell resolution. Advances in 'spectral' flow cytometry have dramatically improved the ability to discriminate signals from different fluorophores, expanding the number of proteins one can detect simultaneously to >30 (ref. 3). Mass cytometry captures even more protein markers on individual cells than flow cytometry, and it is based on a similar approach, although using antibodies tagged not with fluorophores but with heavy metals[4]. When attached to cells, heavy metals are quantified by a mass spectrometer at single-cell resolution, providing high-dimensional single-cell analyses. scRNA-seq captures RNA from individual cells[5], typically using individual lipid droplets, yet the analysis is often conceptually similar to other cytometry approaches, characterizing individual cell identities based on expression of cellular markers. The addition of oligo-DNA-tagged antibodies to the single-cell RNA-seq workflow enables the quantification of both transcription and cell-surface protein expression[6,7]. Data from each of these analyses is often visualized in two-dimensional UMAP plots, and clustering approaches have been the go-to method of quantifying the abundance of the various cell populations within a sample[8,9]. Several methods have been developed to evaluate high-dimensional cellular profiling data to identify differences between two or more patient groups or to associate these data with clinical or serological parameters[10–12].

Distinct immune-cell features across rheumatic diseases

Although standard laboratory tests currently used in clinical practice provide limited insight into immune activation, they already indicate some clear patterns of immune abnormalities across rheumatic diseases. The serum CRP level is characteristically elevated in giant cell arteritis and polymyalgia rheumatica (PMR), reflecting an important role for IL-6, which induces CRP expression, in these diseases[13,14], yet CRP levels are often normal during SLE flares[15,16]. By contrast, serum complement levels are reduced in active SLE but not in giant-cell arteritis or PMR, reflecting the immune complex formation and complement

Box 1 | Features that reveal heterogeneity in patients with rheumatoid arthritis

Clinical heterogeneity
- Demographic characteristics
- Joint distribution
- Extra-articular disease manifestations
- Erosion extent[150]
- Imaging heterogeneity (in ultrasonography)[151]

Serological and transcriptomic heterogeneity
- Erythrocyte sedimentation rate and levels of C-reactive protein
- Seropositivity for autoantibodies such as anti-CCP and rheumatoid factor
- Multi-analyte immunoassays
- Cytokine signatures
- Additional autoantibodies

Genetic heterogeneity
- HLA alleles[152]
- Non-HLA risk alleles[153]
- Polygenic risk scores[154,155]

Histological heterogeneity
- Krenn histological scores[156]
- Cellular density
- Immune-cell aggregates
- Pathotype: lympho-myeloid, diffuse-myeloid, or fibroid[64]
- Ectopic lymphoid structures

Cellular heterogeneity in the synovium[39,113]
- Cell-type abundance phenotypes: T cells+B cells (T+B); T cells+myeloid cells (T+M); T cells+fibroblasts (T+F); myeloid cells (M); fibroblasts (F); endothelial cells+fibroblasts+myeloid cells (E+F+M)[39]
- T cell phenotypes: T peripheral helper and T follicular helper cells, granzyme K^+ T cells, granzyme B^+ T cells, regulatory T cells[39,42,50]
- B cell infiltrates: age-associated B cells, plasma cells[39]
- Myeloid phenotypes: $HBEGF^+IL1B^+$, $SLAMF7^+$, $MERTK^+$ macrophages[39,113,157], conventional type 2 dendritic cells, and inflammatory dendritic type 3 cells[114]
- Fibroblast phenotypes: lining fibroblasts, sublining fibroblasts, perivascular fibroblasts[39,158,159]

Cellular heterogeneity in the blood
- T cell phenotypes: T peripheral helper or T follicular helper cells, effector-memory T cells that re-express the naive-cell marker CD45RA, regulatory T cells, T helper 17 cells[42,69]
- B cell phenotypes: age-associated B cells, plasmablasts[160]
- Myeloid phenotypes: monocytes, dendritic cells[161]
- Pre-inflammatory mesenchymal cells[108]

cascade activation in SLE[2]. Early microarray studies highlighted a prominent upregulation of interferon (IFN)-stimulated genes in blood cells of patients with SLE, far exceeding levels seen in patients with inflammatory arthritis[17]. This recognition of a prominent activation of a type I IFN response in SLE fuelled the evaluation and ultimate approval of type I IFN receptor (IFNAR) blockade with anifrolumab to treat SLE[18]. SLE provides a benchmark for strong activation of a type I IFN response against which other diseases can be compared[19]. Although type I IFN signatures are not routinely measured, commercial tests that quantify these signatures are becoming available[20].

Moving beyond cytokine signatures, immune-cell profiling studies are now identifying some of the major axes of immune activation that distinguish autoimmune rheumatic diseases or disease groups with cellular resolution. Flow-cytometry profiling of blood cells from almost 1,000 individuals, representing 11 autoimmune rheumatic diseases, revealed characteristic patterns of activation in immune-cell subsets across diseases. SLE and mixed connective tissue disease (MCTD) showed shared patterns, as expected given their clinical and serological similarities, whereas RA and spondyloarthropathies (SpA), including psoriatic arthritis (PsA) and axial spondyloarthritis (axSpA), shared a distinct set of immune features, perhaps reflecting the shared responsiveness of RA and SpA to inhibitors of TNF[21]. SLE and MCTD were associated with a particularly prominent expansion of activated ($HLA\text{-}DR^+ CD38^+$) $CD4^+$ and $CD8^+$ T cells in circulation, consistent with observations from other studies[22–24]. Additional studies have highlighted a T cell–B cell axis, involving expansion of both B cell-helper T cells, such as T follicular helper (T_{FH}) cells and T peripheral helper (T_{PH}) cells, as well as activated B cells, as a core immunological feature of SLE[25–31]. This T cell–B cell axis stands out in blood immune profiles of patients with SLE when these are compared with patients with other rheumatic diseases; blood profiles of patients with RA do not show the same extent of adaptive immune-cell dysregulation on average as seen with patients with SLE[25,32,33].

Combining cellular and transcriptomic profiling, the ImmunoNexUT consortium reported bulk RNA-seq transcriptomes of 28 sorted immune-cell populations from the blood of 337 individuals with ten rheumatic diseases[34]. With this broad approach, diseases segregated into two major groups: immune profiles of SLE, MCTD, RA and systemic sclerosis (SSc) segregated from those of the autoinflammatory conditions Behcet disease and adult-onset Still's disease. Correspondingly, IFN signatures were enriched in SLE and MCTD, as well as in some SSc, idiopathic inflammatory myopathy and Sjögren disease, whereas IL-18 or IL-1 signatures were enriched in Behcet disease and adult-onset Still's disease[34].

Rheumatoid arthritis versus spondyloarthritis

RA and PsA represent similar, but distinct, forms of inflammatory arthritis, with distinguishable patterns of joint involvement, risk factors, demographics and genetics. Clinical trials have highlighted differences in the efficacy of various immunological therapies for these conditions, with IL-17A blockade being more efficacious in SpA than in RA, despite similar total levels of expression of IL-17A in synovial samples[35]. By contrast, B cell depletion with rituximab is commonly used to treat RA but has not shown clear efficacy in SpA[36,37]. These treatment response differences illustrate that immunological drivers differ between these conditions; immune-cell profiling studies are now providing a clearer view of the cellular immunology that underlies these therapeutic differences.

Immune cells in synovial tissue or synovial fluid have been evaluated by scRNA-seq and mass cytometry in both RA and PsA[24,38–41].

T_{PH} cells were markedly expanded in joints of patients with seropositive RA but had comparatively lower abundance in patients with seronegative RA or SpA[24,42,43]. T_{PH} cells are a subset of CD4 T helper cells specialized to provide help to B cells, much like T_{FH} cells; T_{PH} cell expansion in seropositive RA is aligned with the roles of T_{PH} cells in B cell recruitment and stimulation[44]. By contrast, IL-17A$^+$ CD8 T cells[45,46] are enriched in the joints of patients with PsA, and this finding is consistent with the responsiveness of these patients to IL-17A blockade[47,48]. In addition, profiling of T cells from synovial fluid of patients with axSpA showed increased abundance of an integrin-expressing CD103$^+$ CD49a$^+$ CD8 T cell population that expressed both IL-17A and cytotoxic molecules[49]. The distinct patterns of expanded T_{PH} cells in seropositive RA versus IL-17A$^+$ CD8 T cells in SpA seem to align well with the differential efficacy of B cell depletion versus IL-17A blockade in these two diseases. Nevertheless, other features, including accumulation of granzyme K (GZMK)$^+$ CD8 T cells, granzyme B (GZMB)$^+$ cytotoxic T cells, and regulatory T (T_{Reg}) cells within joints, are shared between RA and SpA[38,50]. Further studies are needed to associate lymphocyte features with differences in synovial pathology between RA and PsA, including differences in the patterns of vascular remodelling and immune–stromal interactions[51–53].

Immune-cell profiling studies using blood samples have also indicated distinct circulating immune-cell patterns in RA and PsA. Consistent with results from synovial tissue and synovial fluid, T_{PH} cells are increased in the circulation of patients with seropositive RA, but not seronegative RA or PsA[42,43]. Broad mass cytometry profiling comparing peripheral blood mononuclear cells (PBMCs) from patients with RA or PsA highlighted increased frequencies of terminally differentiated (CD27$^-$ CD28$^-$) effector CD8 T cells in seropositive RA but not in seronegative RA or PsA[54]. Interestingly, the blood-immune profiles of seronegative RA and PsA had no clear differences[54,55]. The large-scale effort of the 'Accelerating Medicines Partnership (AMP) on Autoimmune and Immune-Mediated diseases' (AMP-AIM) network, which includes a comparison of blood and tissue immune profiles between RA and PsA, will provide substantial power to define robust immunological differences distinguishing these diseases, also with spatial resolution within tissues[56]. Although the presence or absence of autoantibodies provides a foundational tool helping to distinguish clinically overlapping entities of seropositive RA, seronegative RA and SpA, one can imagine that immunological assessment of T_{PH}, T_{FH} or T_H17 cell pathways in patients might help to further distinguish subsets of patients with undifferentiated arthritis or patients with seronegative RA to guide selection of an RA- versus an SpA-aligned treatment framework.

Immune checkpoint inhibitor-induced arthritis

Cellular profiling of the active immune response in patient samples has proven valuable in assessing a form of inflammatory arthritis that has emerged with the advent of immunotherapies for the treatment of cancers — immune checkpoint inhibitor (ICI)-induced arthritis. ICI therapy using an antibody that blocks the inhibitory receptor PD-1 induces a range of immune-related adverse events, including ICI-induced inflammatory arthritis, which occurs in ~4% of treated patients[57,58]. ICI-induced arthritis can involve RA-, PsA-, or PMR-like manifestations, usually without generation of anti-cyclic citrullinated peptide (anti-CCP) or rheumatoid factor autoantibodies[58,59]. Similar to RA and PsA, ICI-induced arthritis involves an active, presumably autoreactive, T cell response, yet the specific features of this response differ starkly across the three conditions[24,60]. Mass cytometry-based comparison of T cells from synovial fluid of patients with ICI-induced arthritis, RA or PsA showed clear expansion of a population of CD38^{hi} CD8 T cells specifically in ICI-induced arthritis[24]. CD38^{hi} CD8 T cells were also expanded in the circulation of patients with ICI-induced arthritis, and broadly among patients treated with ICIs, yet these cells were not highly expanded in patients with RA or PsA[24]. Transcriptomic comparisons of synovial fluid T cells demonstrated a higher type I IFN response signature in T cells from patients with ICI-induced arthritis than synovial T cells from patients with RA or PsA, and in vitro treatment of synovial fluid CD8 T cells from patients with RA or PsA with type I IFN promoted acquisition of the CD38^{hi} T cell phenotype seen in patients with ICI-induced arthritis. The type I IFN signature in ICI-induced arthritis samples provided an unexpected immunological link between ICI-induced arthritis and SLE, a disease marked by high type I IFN production that also features expanded CD38^{hi} CD8 T cells[19,23].

Defining such immunological benchmarks across diseases is likely to provide a deeper understanding of why certain therapies work well in one condition versus another and might help to identify therapies that are likely to work in newly emerging conditions, such as those induced by immunotherapies. Cross-disease comparisons integrating data across different forms of inflammatory arthritis, including juvenile idiopathic arthritis and others[61], should demonstrate the relative prominence of specific features of the active immune response in inflamed joints, including the abundance of proliferating, exhausted or stem cell-like lymphocytes, the expansion of T_{PH} and T_{FH} cells, the presence of GZMK$^+$ T cells versus GZMB$^+$ T cells, and the frequencies of T_{Reg} cells, infiltrating monocytes versus tissue-resident macrophages, dendritic-cell (DC) populations, age-associated B cells (ABCs) and plasmablasts, to amass a clearer taxonomy of inflammatory arthritides according to features of immune activation[62].

Immune-cell heterogeneity within a disease

Immune-cell heterogeneity in rheumatoid arthritis

In addition to highlighting differences across diseases, immune-cell profiling is a valuable tool for dissecting immunological heterogeneity among patients who share a diagnosis. Patients with RA display substantial variability in clinical course, the likelihood of developing erosions and response to treatments. Correspondingly, studies of synovial tissues have highlighted differences in synovial immune infiltrates among patients with RA, even when they share comparable imaging and clinical features of synovitis[63,64]. Patients with seropositive RA frequently show a 'lympho-myeloid' pattern of immune infiltration in the inflamed synovium, with aggregates of synovial B cells and T cells that range from loose, disorganized clusters to well-organized follicular structures[65]. In other patients with RA, the synovium either shows a diffuse myeloid-cell infiltrate without lymphoid follicles or a 'fibroid' or 'pauci-immune' synovial pattern with few immune-cell infiltrates. Patients with a lympho-myeloid pathotype are the most likely to develop erosions and joint damage progression, whereas patients with a fibroid pathotype show the lowest disease activity, yet also the weakest response to DMARD treatment[66]. Detailed cellular analyses have defined the composition of immune cells in synovial-tissue samples across the various pathotypes. scRNA-seq of RA synovial biopsies delineated six 'cell-type abundance phenotypes' (CTAPs), representing six types of synovial inflammation, that differ in the relative abundance of each of the following cell types: fibroblasts; T cells and NK cells; B cells; endothelial cells; and myeloid cells[39] (Box 1). These CTAPs roughly correspond to histological patterns, with the CTAP containing both T cells and B cells (CTAP-TB) showing the highest histological scores of synovitis (according to the histological score developed by Krenn) and aggregate density. However, immunological information captured by

CTAPs largely seems to be orthogonal to clinical or serological assessments, suggesting that these tissue analyses will be complementary, and not redundant, with current clinical assessment of RA.

Given the difficulties of sampling synovial tissue from patients with RA, there has been substantial interest in identifying signatures in blood that capture immune activity in the joints. Direct parallels between synovial infiltrates and immune-cell phenotypes in blood are challenging to identify, although some shared features of the adaptive immune response have been demonstrated, such as shared T cell receptors (TCRs) and, occasionally, shared T cell clone phenotypes in synovium and blood of patients with RA, PsA and ICI-induced arthritis[24,50,67,68]. Analyses of paired blood and tissue samples from large numbers of patients, such as those profiled in the AMP RA/SLE Network, should help to clarify the extent to which features of immune cells in blood can reflect specific immune processes occurring within synovium.

Independently of synovial analyses, flow cytometry profiling of blood cells from over 500 patients with RA has highlighted substantial variability in immune-cell profiles that were non-redundant with clinical and serological phenotypes[69]. These blood immune profiles were used to stratify patients into peripheral blood-cell abundance phenotypes (PCAPs, analogous to synovial CTAPs). Patients with distinct PCAPs showed distinct patterns of cell abnormalities, including one group of patients with expanded activated CD4 T cells, CD8 T cells and plasmablasts (PCAP-TB), a separate group with increased effector-memory T cells that re-express the naive-cell marker CD45RA (T_{EMRA} cells) or T_{EMRA} and T_H1 cells (PCAP-T1/T1T4), and two more patient subgroups (PCAP-LD and PCAP-SD) that cytometrically resembled healthy individuals[69]. The frequency of anti-CCP antibody or rheumatoid factor did not differ across these groups, yet patients in the PCAP-TB group showed the highest disease activity and ESR, as well as the least frequent use of methotrexate. Inclusion of additional immune-cell subsets with an emerging role in disease pathogenesis, including T_{PH} cells, T_HA cells – a $CXCR3^{mid}$ cytotoxic CD4 T cell population expanded with age[70] – and $GZMK^+$ T cells[50], might enhance the utility of blood-cell profiling in RA. In addition, the identification and quantification of immune-cell subsets are aided by high-resolution scRNA-seq and mass cytometry analyses that precisely define the phenotypes of activated cells in the circulation[71,72]. In total, cellular profiling of blood and tissue samples from patients with RA is providing an additional set of informative variables with which to understand immunopathology in individual patients (Box 1).

Immune-cell heterogeneity in systemic lupus erythematosus

Patients with SLE display stark variability in terms of organs affected, disease severity and response to immunosuppressive therapy, potentially reflecting substantial immunological heterogeneity. Serum proteomics, gene-expression profiling and flow-cytometry analyses have illustrated key features of immune activation in SLE that are consistently observed across cohorts. Expression of IFN-stimulated genes has reproducibly been found to be increased across many SLE studies, with the majority of patients showing a type I IFN signature[17,19]. scRNA-seq profiling has further refined immune-cell populations with the highest expression of an IFN response signature in the blood, including monocyte and lymphocyte subsets[73,74], and has demonstrated a clear IFN response signature across many tissues, including skin and kidney, in SLE[75–78]. In both kidney and skin samples, a subset of T cells and B cells shows a very high IFN signature, above the basally elevated IFN signature seen broadly in cells from patients with SLE compared with healthy individuals[75–77]. What distinguishes the IFN signature-high cells from other cells in the tissue remains unclear. It will be interesting to integrate these observations with emerging spatial transcriptomics data, which suggest that cells with the highest IFN signatures are enriched in the glomeruli in the kidneys of patients with lupus nephritis (LN)[79].

In addition to the IFN signature, other immune signatures extracted from whole-blood transcriptomic analyses have enabled patient stratification into subgroups, particularly when analyses were run on longitudinal samples. Longitudinal whole-blood profiling of patients with childhood-onset SLE stratified patients into seven groups that vary in transcriptomic signatures associated with erythropoiesis, IFN response, myeloid cells and neutrophils, plasmablasts and lymphocytes. Among these patient subgroups, a plasmablast-associated signature was strongly associated with disease activity over time[80]. Studies using blood-transcriptomic profiling of adult patients with SLE have stratified patients into 3–7 subgroups based on similar but not identical features to those used for the stratification of paediatric patients[81–83]. In the adult cohorts, increased expression of inflammation, myeloid/neutrophil and plasmablast transcriptomic signatures have been associated with increased disease activity, as defined based on SLE Disease Activity Index scores[81–83].

The cellular resolution of cytometric profiling studies has in some cases extended understanding of the immune pathways previously implicated by bulk RNA-seq in SLE, for example, the plasmablast-associated signature. Cytometric studies evaluating B cell phenotypes in SLE have extended the understanding of the activated B cell response, which includes expansion of both plasmablasts and ABCs (also known as DN2 B cells), which are characterized by high expression of CD11c and TBET and low CXCR5 and CD21 (refs. 29–31,84). The expansion of $CD21^{low}$ ABCs is perhaps the most prominent cytometric abnormality among circulating B cells in patients with SLE and is highest in patients with active disease, including patients with LN[25,29,30,71,85].

Cytometric profiling can also capture immunological features that are difficult to detect in whole-blood-transcriptomic analyses, for example, the abundance of specific T cell subsets or T cell functional states. Flow cytometry-based profiling of PBMCs stratified patients with SLE into three subgroups based on T cell profiles, with one group marked by expanded T_{FH} cells and activated T_H1 cells (that probably included T_{PH} cells) and a second group marked by expanded T_{Reg} cells[86]. Disease activity or duration did not differ across the three groups, yet the T_{FH} cell-associated group had the highest total immunoglobulin levels, consistent with amplified T cell–B cell interactions. Mass cytometry-based profiling of T cells from patients with SLE highlighted a prominent expansion of T_{PH} cells in patients with LN, with the expansion of circulating T_{PH} cells exceeding that of T_{FH} cells[25]. Both T_{PH} cells and T_{FH} cells have been identified as expanded in multiple cohorts of patients with SLE and associated with the clinical and serological measures of disease activity[26–28,71,87,88]. T_{FH} cell expansion seems to be clearer among patients with shorter disease duration compared with those with longer disease duration[87]. The abundance of T_{PH} cells correlates positively with that of ABCs in patients with SLE, probably reflecting an ongoing extrafollicular response[25,89,90].

The AMP RA/SLE network used mass cytometry of PBMCs to stratify patients with LN into three immunologically distinct subgroups[71]. Among patients with biopsy-demonstrated class III, VI or V nephritis, more than half of whom had established disease with prior treatment for LN and prior kidney biopsies, cytometric profiling identified one subgroup that was immunologically indistinguishable from healthy

individuals, a second subgroup had a very high type I IFN response signature, and a third subgroup had an intermediate type I IFN response signature but a distinctive expansion of GZMB$^+$ T cells, suggesting activation of a 'cytotoxic lymphocyte' axis. Both the type I IFN response-high and GZMB$^+$ subgroups had expansion of proliferating B cells and T_{PH} cells, indicating a shared activation of a B cell–T cell axis. These patient subgroups had distinct features in terms of both kidney histopathology and clinical course; the GZMB$^+$ subgroup had patients with the highest disease activity in the kidney based on the histological NIH activity index and the highest likelihood of a good renal response to standard-of-care therapy at 1 year[71]. By contrast, the immunologically quiet subgroup showed the highest degree of chronic kidney damage histologically, which perhaps reflects prior immunological injury. The poor response to treatment in this subgroup suggests that these patients might have chronic kidney disease without ongoing immune activation and might not benefit from escalated immunosuppressive therapy. Notably, kidney biopsies shared the specific features of blood-immune profiles; patients with a high proportion of GZMB$^+$ T cells in blood also had an increased proportion of GZMB$^+$ CD8 T cells in kidney tissue, and patients with the highest type I IFN signatures in blood also showed the highest IFN signatures in cells from the kidney[71]. Further validation of these signatures and additional prospective studies are needed to determine if a very high type I IFN response signature enriches for patients most likely to respond to IFN blockade, or if cytotoxic T cell activation is differentially susceptible to the various SLE therapies.

Given the observations from studies on RA and SLE discussed above, cytometric immune profiling has the potential to identify immunologically distinct subgroups of patients in other rheumatic diseases as well. In PsA, blood-cell profiling by flow cytometry highlighted four subgroups of patients through principal components analysis, with a subgroup that was characterized by increased frequencies of T_H17 cells, memory T_{Reg} cells, DCs and monocytes being associated with increased disease duration and activity[91]. Moreover, scRNA-seq analysis of blood segregated patients with Sjögren disease into two major subgroups, corresponding to the presence or absence of anti-SSA antibodies; a strong type I IFN signature was associated with anti-SSA seropositivity[92]. Integrating large datasets, especially scRNA-seq datasets, across diseases might provide the ability to identify immunologically similar patients across clinical-disease presentations.

Immune profiling of treatment responses

In both RA and SLE, the expanded armamentarium of immunosuppressive drugs poses new challenges for patients and physicians in selecting which therapy is most likely to be beneficial for an individual patient. In RA, at least five mechanistically distinct classes of biologic therapies are available: TNF blockade; IL-6 blockade; JAK inhibition; B cell depletion; and T cell costimulation blockade[93]. However, there is little guidance on the decision about which therapy to use for an individual patient. In SLE, the expanded range of treatment options, now including B cell inhibition or depletion[94], IFNAR blockade[18] and calcineurin inhibition[95], similarly poses questions about which drug to use for which patient. Longitudinal studies of pre- and post-treatment samples provide crucial insights into the major pathways affected by each DMARD and potentially identify cellular features at baseline that are associated with a good response to treatment. This review will not attempt to broadly summarize the wide range of cellular treatment response biomarker studies in RA and SLE, but will, rather, highlight specific examples of promising approaches or consistently observed signals.

Profiling treatment responses in rheumatoid arthritis

Blood-cell-based profiling of treatment responses. Identifying predictors of patient responses to DMARDs remains an area of active research in RA. Tremendous effort has been focused on identifying biomarkers of response to TNF inhibitors, but analyses of standard laboratory markers, antibodies, serum proteins, whole-blood transcriptomes and cell phenotypes have not yet led to the identification of any robust predictors of treatment responses[96,97]. The advent of broad profiling methodologies has yielded some successes: whole-blood-transcriptomic analyses combined with advanced computational approaches have led to the commercial development of a test to predict the likelihood of a non-response to TNF inhibitor therapy[98,99].

The search for treatment response biomarkers has been importantly advanced by applying immune-profiling studies within the context of clinical trials, especially those involving randomization. This approach leverages the standardized clinical assessment of disease activity within a trial infrastructure, and the randomization minimizes concerns about confounding by indication and unaccounted bias. Such studies have highlighted a reproducible relationship between the frequency of circulating T_{FH} cells in the blood and response to abatacept, a drug that blocks T cell costimulation. In a cohort of patients analysed by flow cytometry in the NORD-STAR trial, which randomized patients with early RA to methotrexate plus one of four biologics, cytometric quantification of 12 T cell populations demonstrated a specific association between elevated baseline PD1$^+$ T_{FH} cell frequency and achieving remission following treatment with abatacept[100]. Similarly, in a prospective observational study of patients with RA and an inadequate response to methotrexate, patients who achieved remission after treatment with abatacept had higher frequencies of PD1$^+$ T_{FH} cells in the blood at baseline than patients who did not achieve remission[101]. Consistently, elevated frequencies of activated T_{FH} and T_{FH} cells in the blood of patients with early type 1 diabetes were associated with a good clinical response to abatacept[102]. Abatacept robustly reduces the frequency of circulating T_{FH} and T_{PH} cells, supporting the biologic plausibility of the cellular association with treatment response[101,102].

Studies looking for cytometric features predictive of response to rituximab have highlighted an association with circulating B cell populations. In the SMART trial of rituximab in patients with RA, flow cytometric analysis of B cells indicated that a low proportion of circulating CD27$^+$ memory B cells was associated with a good response to treatment at 24 weeks by EULAR criteria[103]. Independently, the FIRST study, which evaluated 154 patients with RA who were treated with rituximab using flow cytometry, associated a high proportion of circulating CD27$^-$ IgD$^-$ B cells with a good response to rituximab, especially when considered in combination with rheumatoid factor positivity. Combined with additional studies[104,105], these observations strongly associate features of B cell activation or B cell memory with likelihood of response to rituximab. Irrespective of B cell phenotype, a randomized study of 25 patients with RA associated the detection of residual circulating B cells after two doses of rituximab with significantly improved response rates to a third dose of rituximab[106].

Applying standardized profiling methods across patients treated with various DMARDs has the potential to identify specific cellular patterns that are associated with an improved response to a specific treatment. Exploratory studies using flow-cytometry profiling, applied longitudinally to over 500 patients with RA as described above, identified subgroups of patients with a differing likelihood of response to the various DMARD classes[69]. Using PCAPs to stratify patients as

described above and prospective longitudinal evaluation indicated that patients in the PCAP-TB group, who are marked by an active B cell response, were the least likely to achieve remission overall after treatment with one of four biologic DMARDs interrogated: abatacept, JAK inhibitors, TNF inhibitors and IL-6 inhibitors. By contrast, patients in the PCAP-SD or PCAP-LD groups, who collectively showed relatively few cellular changes compared with healthy individuals, were more likely to achieve remission following treatment with JAK inhibitors than were patients in other PCAPs.

To define operational links between PCAPs and treatment assignments, the authors then assigned each of the four specific DMARDs as associated or not associated with a good response for each PCAP-based patient subgroup. Patients were then assigned a status of 'expected' or 'non-expected', reflecting whether the patient received a DMARD associated with a good response in their identified PCAP. Promisingly, in a validation cohort of 183 patients, patients with an 'expected' designation, indicating that the patient received a DMARD expected to produce a good response in their identified PCAP, were more likely to achieve remission than patients with a 'non-expected' treatment assignment (33% versus 18%). These treatment-response associations need to be validated further, and a substantial challenge remains to identify stratification parameters that can be reproduced and adopted widely. Nonetheless, the impressive scale of the study and the ability to reproduce signatures in a validation cohort provide hope for extension of this approach. A broader immune profiling approach that captures activated T_{FH} and T_{PH} cells or other cell populations with a key role in RA might improve treatment assignment to specific patient subgroups.

Using a clever, alternative strategy, the BioRRA study investigated how circulating immune-cell profiles change during arthritic flares that occur in patients with RA after withdrawal of synthetic DMARD treatment[107]. The analyses associated expansion of activated T cell and B cell populations, including $PD1^+$ $CD38^+$ CD8 T cells and $PD1^+$ $ICOS^+$ $CD38^+$ CD4 T cells, with disease flares after DMARD withdrawal. This finding suggests that synthetic DMARDs hold these T cell populations in check, such that treatment withdrawal allows for $PD1^+$ $CD38^+$ CD8 T cell and $PD1^+$ $ICOS^+$ $CD38^+$ CD4 T cell activation and expansion. Frequencies of these cell populations at baseline (pre-drug withdrawal) did not differ between patients who remained in remission and patients who experienced disease flares after drug withdrawal; thus, it is unclear whether such signals can help predict disease relapse prior to drug withdrawal. Nonetheless, the above cellular correlates might provide a valuable readout to confirm re-activation of the disease-associated immune response, if symptoms emerge following treatment cessation. Immune-cell profiles that are potentially associated with disease flares were also identified by a separate study using frequent, serial assessment of whole-blood samples by RNA-seq; in this study, disease flares were associated with preceding changes in B cell signatures and a concurrent increase in rare, circulating mesenchymal cells potentially related to synovial fibroblasts, called PRIME cells, during the flare[108].

Synovial cell-based profiling of treatment responses. There is major interest in understanding the associations between immunological features in synovium and response to the various treatments. Results from the pioneering R4RA trial provided encouraging initial observations, indicating that patients with a diffuse myeloid infiltrate were more likely to respond to tocilizumab than to rituximab[109]. Extending these observations using CTAP designations further supported the idea that patients with a fibroid (CTAP-F) phenotype, generally lacking large lymphocyte or myeloid infiltrates, were the least likely to respond to biologic treatment[39,110]. One tangible prediction in connecting synovial infiltrates to treatment response would be that patients with a B cell-enriched synovium are more likely to respond to B cell depletion with rituximab than patients without B cells in synovium; however, this has not been consistently observed in the clinical trials that have assessed synovium[109,111,112]. Among synovial myeloid cells, an increased proportion of $MerTK^+$ tissue macrophages is associated with a state of treatment-induced remission in RA, and an increased proportion of $MerTK^+$ tissue macrophages in synovium at baseline is associated with maintenance of remission after TNF inhibitor withdrawal[113]. Spatial transcriptomic analyses have further associated synovial DC populations with disease activity and treatment response, reporting on a tolerogenic AXL^+ cDC2 population that is present in healthy synovium but absent in RA synovium, even when remission is achieved, suggesting a lasting remodelling of the DC populations due to synovitis[114].

With these early observations guiding new study design and analysis approaches, there remains substantial enthusiasm that cellular features within synovium will provide crucial insights into the variable treatment responses of patients with RA. Detailed single-cell analyses, including spatial transcriptomic analyses, comparing pre-treatment and post-treatment samples, as reported in inflammatory bowel disease, should aid in identifying specific cell populations associated with response and non-response to treatment[115]. Indicatively, spatial transcriptomic analyses of longitudinal synovial tissue biopsies from patients with RA demonstrated a $COMP^{hi}$ fibrogenic fibroblast population that is enriched in pre-treatment samples of patients who do not achieve remission and that persists in synovium despite effective reduction of immune cells by DMARD therapy[56].

Profiling treatment responses in systemic lupus erythematosus

Cellular or molecular signals that are associated with treatment effects and treatment responses have been identified in several clinical trials in SLE. Correlative transcriptomic and serum-profiling studies of patients treated with anifrolumab have illustrated a clear reduction in IFN responses at both transcriptomic and proteomic levels in treated patients in both clinical trials and observational studies[116–118]. Profiling of blood samples from patients with SLE before and after treatment with anifrolumab in the MUSE trial demonstrated that anifrolumab alters several measures of immune activation in SLE, with a high IFN signature at baseline; anifrolumab treatment increased numbers of circulating neutrophils, platelets and lymphocytes, especially naïve CD4 and CD8 T cells[118]. Anifrolumab treatment also reduces circulating levels of several chemokines, including CXCL13, a potent B cell chemoattractant produced by T_{PH} and T_{FH} cells[118,119]. Longitudinal scRNA-seq profiling of blood samples from a small cohort of patients that received anifrolumab demonstrated that IFNAR blockade reduces the abundance of circulating T_{PH} cells, and concurrently expands a counter-regulated population of IL-22-producing CD4 T cells (T_H22 cells), which are reduced in patients with active SLE[119]. This reduction in circulating T_{PH} cells following type I IFN blockade functionally links IFN signalling to enhanced T cell–B cell interactions and B cell activation in SLE[119]. Understanding the effects of type I IFN blockade on other components of the pathological adaptive immune response in SLE is of major interest. Thus far, it has not been evident from available data that patients with a low IFN signature have a substantially weaker clinical response to

Table 1 | Immune profiling of response to treatment

Type of treatment	Engagement of primary target	Pre-treatment vs post-treatment comparisons	Predictors of treatment response	Assessment of treatment duration
Synthetic DMARDS	Unclear	What cell populations or pathways are most altered by treatment?	What cellular features at baseline (pre-treatment) predict a good response to treatment?	For how long can therapy continue to suppress signs or markers of immune dysregulation?
Biologic DMARDs	Inhibition of targeted pathway (for example, TNF, IL-6, IFN)			
CAR T cells or depleting antibodies	Depletion of targeted cell population (for example, B cells, plasma cells, $PD1^{hi}$ T cells)			For how long does cell depletion last? For how long do signs or markers of immune dysregulation remain absent after a single dose of the respective treatment?
BiTEs	Depletion of targeted cell population Extent and nature of T cell activation			

BiTEs, bispecific T cell engagers; CAR T cells, chimeric antigen receptor T cells; IFN, interferon; PD1, programmed cell death protein 1.

anifrolumab than those with a prominent IFN signature at baseline[120]; further immunological assessments might help to dissect whether specific features of the IFN response, such as very prominent and distinctive IFN activation[71] or expansion of IFN-associated immune-cell populations, predict a better response to anifrolumab.

Longitudinal profiling of blood samples from patients treated with B cell-directed therapies have also identified cellular correlates of treatment effect and response. Treatment with belimumab, an FDA-approved agent for SLE that blocks B cell activating factor (BAFF), reduced whole-blood-transcriptomic signatures associated with B cells, as well as signatures associated with IFN and IL-6 signalling and neutrophils, especially in responders[121]. Similarly, patients treated with tabalumab, an IgG4 antibody that blocks BAFF, also demonstrated a reduction in B cell-associated transcripts in whole-blood transcriptomics, consistent with a reduction in circulating B cell counts[122]. Transcriptomic analyses of sorted leukocyte subsets from blood collected before and after treatment with belimumab demonstrated clear effects of belimumab on the transcriptomic features of B cell subsets, with few effects on transcriptomes of circulating T cell or myeloid cell subsets, consistent with the direct effects of belimumab on B cell activation[123]. Further, the number of differentially expressed genes, comparing pre-treatment and post-treatment B cell subset transcriptomes, was higher in good responders to belimumab treatment than in poor responders. A separate study reported reductions in both $CD19^{+}$ B cells and activated $PD1^{+}$ T cells after treatment with belimumab[124]. Interestingly, a longitudinal flow-cytometry assessment of T cell subsets from the blood of patients treated with belimumab demonstrated an increase in the ratio of T_{Reg}–T_H17 cells following treatment, an effect that was reproduced in an independent, broader mass-cytometry profiling study[125,126]. These observations associate specific immune alterations with BAFF blockade, with both direct effects on B cells and secondary effects on T cells.

The use of molecular profiling in studies evaluating new therapeutic agents might also facilitate the identification of molecular predictors of treatment response in SLE. In a phase II trial of obexelimab, a bifunctional antibody that binds CD19 and the inhibitory receptor FcγRIIB, given after initial high-dose steroid treatment, whole-blood transcriptomics were used to classify patients into subgroups: patients with increased expression of lymphocyte modules and cell-proliferation modules but without high expression of inflammation-associated modules were more likely to respond to obexelimab than patients from other subgroups, as assessed by maintenance of disease improvement[127]. In a phase IIb study with iberdomide, a cereblon ligand that promotes degradation of the B cell transcription factors Ikaros and Aiolos, which are important for lymphocyte development and function and which both have polymorphisms associated with SLE[128], blood-cell profiling demonstrated that treatment resulted in dose-dependent decreases in the number of circulating B cells and memory B cells, as well as in plasmacytoid DCs and myeloid DCs[20]. Concurrently, the number of T_{Reg} cells increased in a dose-dependent fashion, paralleling an increase in circulating IL-2 levels. Transcriptomic analyses also highlighted clear reductions in IFN response signature with treatment, and patients with the highest IFN response signature at baseline were the mostly likely to have reduced disease activity after treatment, as assessed by SLE responder index 4 (SRI4)[20].

Following these examples, it is of substantial interest to define the effects of the commonly used synthetic DMARDs, such as azathioprine and mycophenolate, given their widespread use and their difficult-to-predict effects on cellular immunology. The effects of these drugs have not yet been revisited in detail using high-dimensional cellular profiling approaches. Looking forward, understanding the broad scope of immunological changes induced by cell-depletion strategies, such as CD19 CAR T cells and bispecific T cell engagers (BiTEs), will be crucial[129–131]. Deep B cell depletion with these methods has the potential to correct multiple immune abnormalities in patients with SLE, including normalization of complement levels and reduction in type I IFN response[129,132], but the extent to which B cell depletion also corrects T cell and myeloid-cell abnormalities in patients with SLE remains to be defined. The extent and nature of CD8 and CD4 T cell activation induced by BiTEs that target B cells can also be assessed using broad immune-profiling approaches (Table 1).

Immune profiles of individuals with undiagnosed disease

Cellular profiling studies typically utilize a grouped comparison analysis strategy, comparing patients with healthy individuals, pre-treatment with post-treatment, or responders with non-responders. However, cellular profiling also has the potential to identify individual patients with very abnormal features of immune activation compared with a reference population. A pilot study evaluating this approach was performed on samples from 16 patients seen in the Undiagnosed Diseases Network

programme, an NIH-funded programme that focuses on patients with very rare or unusual disease presentations[133,134]. These 16 patients, who all have unusual disease presentations thought to be potentially immune associated, underwent whole-exome or -genome sequencing that did not reveal a clear monogenic cause of disease. This cohort therefore underwent mass cytometry immune profiling of blood cells, and immune profiles were assessed against ~140 reference datasets that included healthy individuals, patients with RA and patients with SLE[134]. Immune profiles from 5 of the 16 patients from the Undiagnosed Diseases Network programme were identified as 'outliers' based on the presence of at least one immune-cell population that was extremely expanded compared with the overall cohort, but no outliers were identified among the reference datasets. Of these patients, one had a dramatic expansion of $CD25^{hi}$ T_{Reg} cells, which comprised 50% of the circulating CD4 T cells, one was identified as having B cell leukaemia, one had an aberrant expansion of a gamma delta T cell population, and one had a very abnormal myeloid-cell phenotype. This exploratory work suggests that immune profiling can be used to identify specific immunological abnormalities in individuals with rare or unusual disease presentations and enable individualized treatment strategies.

Such an immune profiling approach can complement interrogation for rare monogenic causes of immune-mediated disease using whole-genome or whole-exome sequencing[135] or bulk RNA-seq and scRNA-seq analyses reporting outlier gene- or splice-variant expression profiles[136,137]. In both cases, immune profiling has the potential to delineate pathways of immune activation that are activated in the context of a monogenic disease and potentially relevant for treatment. In addition, deep analyses using scRNA-seq might be able to identify immunological abnormalities missed by the cytometric approach, as scRNA-seq captures cytokine response signatures more readily than protein cytometry. As scRNA-seq analyses of PBMCs from healthy donors, individuals at risk of disease (for example, individuals at risk of RA[138,139]) or individuals with defined diseases, including SLE[73], RA, Sjögren disease[92], SSc[140] and others, are becoming increasingly available, they enable mapping of any individual scRNA-seq profile to these reference datasets to identify aberrant cell populations, phenotypes, or states in an individual.

Translation into clinical practice

Cellular profiling studies have yielded several robust features of immune activation or dysregulation that capture clinically relevant information. Assessment of such features in clinical practice might be complementary to and non-redundant with serological tests. Translation of findings from cellular profiling studies into clinical practice could follow multiple paths, but two paths will be considered here: the implementation of flow cytometry-based assessment of pathological immune activation and the introduction of scRNA-seq analysis in a clinical setting.

Cytometric assessment of pathological immune activation

A straightforward path to clinical implementation might involve distilling down the most informative features from high-dimensional profiling studies and then developing targeted, cost-effective tests for these features. In SLE, a disease with prominent immune abnormalities in blood, several informative features from transcriptomic and cytometric studies can be captured in straightforward ways. An IFN signature can be captured by flow cytometric screening for the cell surface marker Siglec-1, a protein strongly upregulated on monocytes by type I IFN[141,142]. The additional four features (proliferating lymphocytes, cytotoxic T cells, $CD21^{low}$ B cells, low-density neutrophils) that stratified subgroups of patients with LN as discussed above could be distilled down to simple parameters that can be measured by flow cytometry[71]. Similarly, the major defining features of synovial CTAPs in RA can be captured by standard flow cytometry[39]. Although, thus far, flow cytometry has little regular use in patients with rheumatic diseases, save for quantifying $CD19^{+}$ B cells in patients treated with anti-CD20 antibodies, this method is routinely used in oncology to aid in the search for haematological malignancies[143]. Further, in clinical immunology, flow cytometry is used routinely to quantify lymphocyte subsets in patients with suspected immunodeficiencies, and also to detect features of immune dysregulation in these diseases, such as expansions of activated B cells and T cells[144].

The development of a flow cytometric test to quantify T_{FH} cell frequency in children with immune dysregulation provides a valuable example of how these tests can be implemented clinically for evaluation of immune activity[145]. Building on established flow-cytometry protocols, an assay to quantify $PD1^{+}$ $CXCR5^{+}$ T_{FH} cells was developed with robust reproducibility across instruments and sample storage times of up to 24 h. Interrogation of cohorts of healthy individuals and individuals with relevant diseases using this assay defined normal ranges and indicated a sensitivity of 88% and specificity of 94% in discriminating autoimmune disease from autoinflammatory conditions[145]. With an estimated cost of <$200 per test, this approach provides a practical, feasible strategy for detection of features of immune activation that are currently missed by routine tests such as ESR, CRP, complement

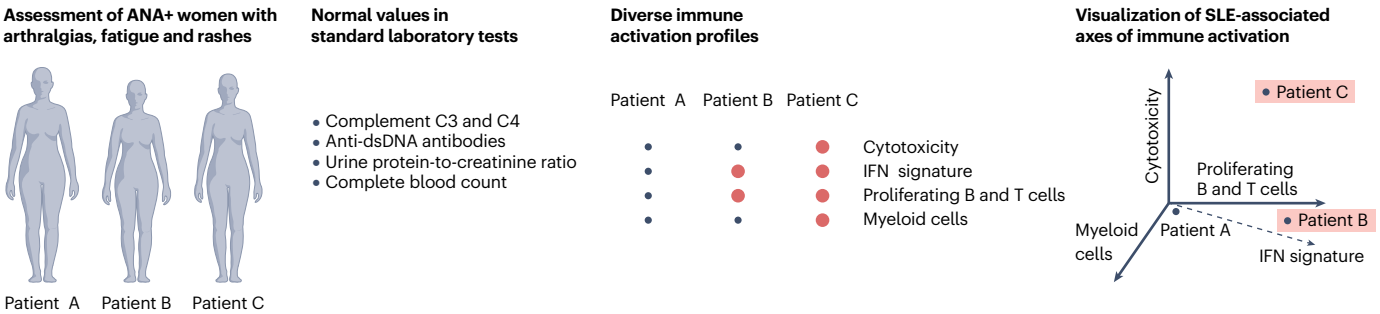

Fig. 1 | Focused immune assessments to identify immune dysregulation in patients with suspected systemic lupus erythematosus. Evaluation of individuals who are seropositive for anti-nuclear antibodies (ANAs) for possible systemic lupus erythematosus (SLE) with standard laboratory tests, followed by immune profiling for specific SLE-associated features of immune dysregulation. In individuals with normal results of standard laboratory tests, immune profiling might reveal SLE-associated immune activity to aid in the diagnosis of SLE. Individual patient profiles can be visualized on axes of immune dysregulation[71]. C3, complement C3; C4, complement C4; dsDNA, double-stranded DNA.

factors and autoantibodies. Although broad adoption of such tests will require standardization of cytometry markers and analysis methods across laboratories, potential value seems clear. For example, for individuals who present at rheumatology clinics with a positive anti-nuclear antibody (ANA), arthralgias, rashes and fatigue but have otherwise normal laboratory tests, a flow-cytometric quantification of circulating T_{FH} cells, T_{PH} cells, ABCs and plasmablasts might help to distinguish between SLE-associated pathological immune activity and immunological quiescence (Fig. 1).

Single-cell RNA sequencing as a clinical tool

Broad profiling approaches such as scRNA-seq of blood samples are likely to be translated into clinical practice in the next decade, following the example of whole-genome sequencing. The rapid advancement of clinical genome sequencing was aided by technological advances that made DNA sequencing feasible at reasonable costs, as well as by the establishment of a reference genome. In this context, immune profiling has struggled with myriad variations in cytometric definitions for the quantification of cell populations, complicating comparison of results

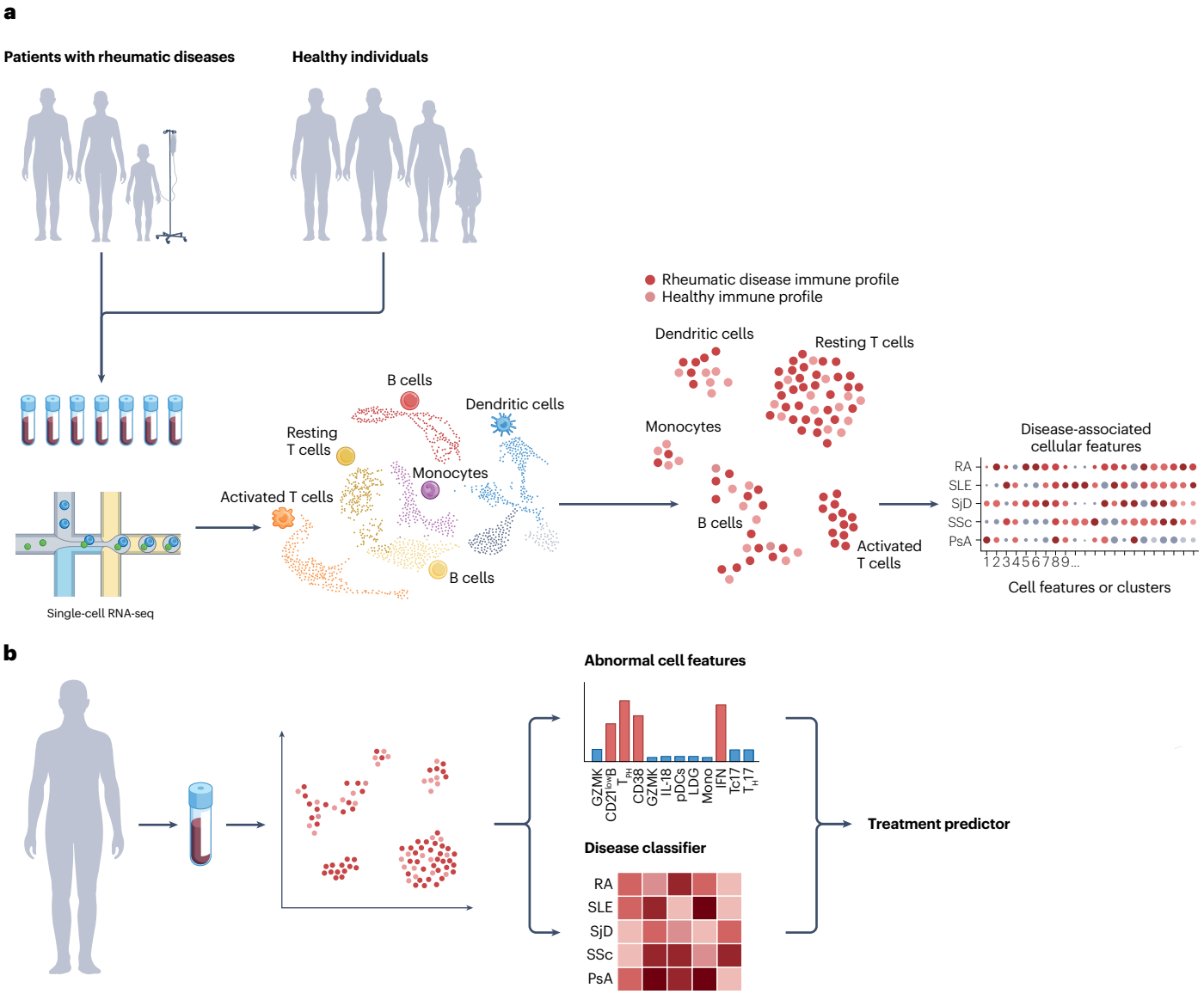

Fig. 2 | Broad immune profiling to identify immune abnormalities in rheumatic diseases. a, Broad immune profiling by single-cell RNA sequencing (RNA-seq) is able to define cell types (for example, monocytes, dendritic cells, B cells, T cells) or cell-activation states (for example, among resting T cells or activated T cells) that are characteristically altered in specific diseases and to generate healthy- and rheumatic-disease-associated reference datasets. These rheumatic-disease-associated cell types or cell states can be considered globally in a multi-dimensional fashion and then delineated further as specific cellular parameters. **b**, The immune profile of an individual sample is mapped against reference profiles to identify cellular features that differ from the healthy control-associated reference. Comparison with rheumatic disease-associated references matches individual profiles of undiagnosed individuals to the most fitting rheumatic-disease reference profile. A combination of these analyses has the potential to identify treatments that are the most suitable to modulate the pathologically activated pathways. LDG, low density granulocyte; Mono, monocyte; pDCs, plasmacytoid dendritic cells; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SjD, Sjögren disease; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

across studies and samples. The widespread use of droplet-based scRNA-seq now provides an opportunity to establish generalizable health- and disease-associated reference datasets. Moreover, advancing computational approaches will enable integration of scRNA-seq results from many diverse datasets, enabling cross-disease comparisons across studies, despite technical and methodological differences[146,147].

Leveraging a common language for scRNA-seq-based immune profiling will enable the mapping of scRNA-seq profiles of individual patient samples against a reference database of health- and disease-defined scRNA-seq profiles (Fig. 2), indicatively, screening for a strong type I IFN signature, as seen in patients with SLE, an expansion of ABCs, plasmablasts, T_{PH} and T_{FH} cells, as seen in SLE, ongoing T cell–B cell interactions, expansion of T_H17 cells, activation of IL-1β or TNF pathways in myeloid cells or abnormal T_{Reg} cell profiles. Unbiased approaches should be able to define SLE-like, RA-like, PsA-like, and other disease-associated immune profiles, enabling an immunophenotypic definition of immune health or disease-like status with any sample. Comparison with other states of immune activation, such as protective anti-viral and antibacterial responses as well as vaccine responses, will also be valuable.

Currently, technical and logistical challenges remain to be overcome for this kind of approach. RNA transcriptomes change with incubation time, such that improved methods are needed that limit artefactual changes in transcriptomic profiles after sample acquisition that may obscure biological signals. Costs of scRNA-seq remain considerable (typically >US$1,000 per sample), slowing the generation of foundational datasets that can demonstrate the utility of such immune profiling, yet newer scRNA-seq profiling methods using probe capture are substantially reducing sequencing costs and broadening the ability to analyse fixed samples[148]. Incorporation of scRNA-seq profiling into the protocols of ongoing industry-sponsored clinical trials, as has been done previously using whole-blood RNA-seq, would be immensely valuable to generate urgently needed scRNA-seq biomarkers of treatment effect and treatment response. As with more focused assays, standardization of methods across laboratories, and agreement on standard reference datasets, will be required to implement these approaches broadly.

Multi-modal immune profiling

A set of cellular profiling assays has the potential to complement other modalities that assess immune or inflammatory features, such as serum proteomic profiling or metabolomic profiling. In some cases, the different modalities might converge on the same fundamental observations; for example, a type I IFN signature can be detected by bulk RNA-seq, PCR, serum proteomic, or cytometric assays in SLE; in this case, the simplest and most cost-effective method should be used. Some modalities might, however, measure a given pathway more efficiently than others; for example, the enzyme-linked immunosorbent assay may be most suitable for detecting a circulating cytokine, mass spectrometry for a key metabolite and cytometry for a relevant cell population. Given the rapid advances in tissue-biopsy profiling with high-dimensional imaging and spatial transcriptomics, specific features of tissue architecture or cell infiltrates, or features of stromal or parenchymal cells, might also provide unique, non-redundant measures of disease-relevant immunopathology. Key informative inputs from any of these modalities can be incorporated as components of a broad assessment of immune dysregulation in patients, adding to the current assessments of CRP, complement components and autoantibodies. Machine-learning approaches that incorporate both molecular and clinical data also have the potential to establish robust diagnostic markers, as in a study that improved identification of patients with PsA using this approach[149].

Conclusion

In total, the rapidly expanding universe of immune-profiling data on blood and tissue samples from patients with rheumatic diseases is providing an increasingly well-defined set of parameters of immune dysregulation that is typical for these diseases, highlighting similarities and differences across diseases and among patients sharing a diagnosis. Immune profiling has so far highlighted several straightforward parameters of immune dysregulation that are ready for clinical implementation. In the near future, broad tests that assess the current activity level of the immune system, with an ability to detect pathological immune activation or deviation from homeostasis, might become as available as blood tests currently used to interrogate the functioning of other organs, such as the kidney and the liver. These methods have the potential to dramatically improve assessment of immune-mediated disease and guide therapeutic decisions for patients with rheumatic diseases.

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Review article

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The essential roles of memory B cells in the pathogenesis of systemic lupus erythematosus

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Abstract

Emerging evidence indicates that memory B cells are dysfunctional in systemic lupus erythematosus (SLE). They are hyporesponsive to signalling through the B cell receptor (BCR) but retain responsiveness to Toll-like receptor (TLR) and type I interferon signalling, as well as to T cell-mediated activation via CD40–CD154. Chronic exposure to immune complexes of ribonucleoprotein (RNP)-specific autoantibodies and TLR-engaging or BCR-engaging cargo is likely to contribute to this partially anergic phenotype. TLR7 or TLR8 signalling and the resulting production of type I interferon, as well as the sustained activation by bystander T cells, fuel a positive feedforward loop in memory B cells that can evade negative selection and permit preferential expansion of anti-RNP autoantibodies. Clinical trials of autologous stem cell transplantation or of B cell-targeted monoclonal antibodies and chimeric antigen receptor (CAR) T cells have correlated replenishment of the memory B cell population with relapse of SLE. Moreover, the BCR hyporesponsiveness of memory B cells might explain the failure of non-depleting B cell-targeting approaches in SLE, including BTK inhibitors and anti-CD22 monoclonal antibodies. Thus, targeting of dysfunctional memory B cells might prove effective in SLE, while also avoiding the adverse events of broad-spectrum targeting of B cell and plasma cell subsets that are not directly involved in disease pathogenesis.

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Introduction

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Key points

- In systemic lupus erythematosus (SLE), memory B cells are hyporesponsive to B cell receptor (BCR) stimulation but can be activated upon engagement of Toll-like receptors (TLRs) and interaction with T cells (mainly via the CD40–CD40L axis). Both innate and adaptive immune signalling by B cells ('bridging') contribute to SLE pathology, possibly via a pathogenic positive feedforward loop.
- This feedforward loop is accentuated by anti-ribonucleoprotein (anti-RNP) autoantibodies sequestering RNP antigens, which, when internalized via the BCR, stimulate TLR7 and TLR8 signalling and type I interferon production.
- Incomplete X chromosomal inactivation of *TLR7*, *TLR8* and *CD40L* might further contribute to such a positive feedforward loop, thereby potentially explaining the female sex bias in SLE.
- Clinical outcomes of B cell depletion in SLE, via anti-CD20 or anti-CD19 or autologous stem cell transplantation, have clearly associated relapse with memory B cell repletion, independently of the recurrence of naive B cells or autoantibodies.
- The safety and efficacy of CD19-targeted and BCMA-targeted chimeric antigen receptor (CAR) T cells, or bispecific T cell engagers in SLE, and their impact on tissue-resident memory B cells remain to be elucidated.
- BCR signalling inhibition approaches did not result in sufficient efficacy potentially owing to an incomplete impact on memory B cells.

Introduction

B cell maturation and B cell activation require highly sophisticated orchestration of molecular processes in the bone marrow and the periphery, respectively. B cell maturation in the bone marrow involves sequential rearrangements of the genes that encode the heavy and light chains of the B cell receptor (BCR). In the periphery, naive B cell activation occurs in secondary lymphoid tissues (spleen, lymph nodes and Peyer's patches) following the stimulation of innate immune cells and T cells by exogenous stimuli. BCR binding often results in antigen internalization and presentation using MHC class II, and the initial steps of B cell activation are supported by signals from appropriately stimulated T follicular helper cells (T_{FH} cells) or T peripheral helper cells (T_{PH} cells)[1]. T cell–B cell collaboration occurs at the T cell to B cell interface of secondary lymphoid organs and requires physical interactions between CD154 (also known as CD40L) and CD40, followed by signalling via the cytokines IL-21, type I interferons, type II interferon (also known as IFNγ) and TGFβ, and later on by IL-6, BAFF and APRIL, which are all important for the survival of B cells and plasma cells.

Effectively activated B cells enter germinal centres within B cell follicles and undergo rapid clonal expansion, immunoglobulin heavy chain class switching, affinity maturation, somatic hypermutation and differentiation into both memory B cells and plasma cells. Vaccination studies in healthy volunteers have provided a basis for understanding B cell activation during primary and secondary immune responses[2,3]. Vaccination is known to lead to the maturation of IgM followed by IgG responses, and selection of high-affinity clones, finally ending with the resolution of the immune response. A successful primary immune response to exogenous antigens requires the tight regulation of naive B cell activation, expansion, somatic hypermutation, differentiation and selection by cytokines, and interactions between T cells, B cells and follicular dendritic cells (FDCs) that take place within the dark and light zones of germinal centres[4]. The germinal centre light zone is an important site for peripheral negative selection of autoreactive B cell clones. Upon secondary vaccine challenges, memory B cells are immediately reactivated and naive B cells are recruited to germinal centres and extrafollicular sites, but these responses resolve within 2–4 weeks. Memory B cells circulate widely in secondary lymphoid organs and tissues, whereas plasma cells largely home to the bone marrow, where they mature into short-lived or long-lived plasma cells that produce antibodies over various periods of time[5]. This highly regulated T cell-dependent B cell activation is essential for immune protection against infections but is simultaneously prone to lymphomagenesis or tolerance breakdown and allergy or autoimmunity. In systemic lupus erythematosus (SLE), many aspects of this process are deranged, resulting in persistent activation and differentiation of effector cells without appropriate resolution.

Although signalling through the BCR is involved in several steps of B cell activation, maturation and negative selection, B cells are additionally regulated by Toll-like receptor (TLR) signalling and CD40 activation by the T helper cell surface molecule CD154. In the context of SLE, the intracellular TLR7 and TLR8 molecules respond to nuclear antigens (such as single-stranded RNA (ssRNA) or U-rich ssRNA) by triggering the production of type I interferon, whereas activated T_{FH} and T_{PH} cells provide B cell survival and differentiation signals[6–8] (Fig. 1).

SLE is an autoimmune disease characterized by profound perturbations of B cell activation and differentiation, which together result in the production of a variety of pathogenic autoantibodies[9]. Numerous abnormalities of B cell function have been documented in patients with SLE[10], and B cell-directed therapies have shown efficacy in some of these patients[11]. Early research in SLE mainly delineated phenotypic differences, including an expanded memory B cell population[12,13] and associated the persistence of subsets of atypical memory B cells, such as $CD27^{-}IgD^{-}$ (double-negative (DN) B cells)[14] and $CD11c^{+}$ age-associated B cells[7,15] (ABCs) that do not rapidly contract as seen following virus infections[16], with disease activity. Later studies found substantial functional and spatial abnormalities in B cell activation and differentiation in SLE[6,17,18]. The initial activation of antigen-naive B cells appears to be dysregulated in SLE, potentially abetted by ineffective negative selection and, therefore, resulting in the enrichment for autoantigen reactivity[19]. Autoreactive memory B cell subsets have been found to differentiate at sites that do not support negative selection processes, such as within extrafollicular sites or in tissues[18,20,21]. Importantly, memory B cells from patients with SLE have shown reduced BCR responsiveness in functional studies, reflecting a profound post-activation anergy[22,23] (Fig. 1). This finding strikingly contradicts textbook knowledge referring to 'hyperactive B cells in SLE', which is often used as a rationale for B cell targeting. In this context, the study of SLE-associated B cells has revived interest in BCR-independent B cell activation pathways, namely TLR7 and CD40 activation, providing the basis for an 'SLE pathogenesis at the crossroad of innate and adaptive immunity' hypothesis.

Based on these developments, we suggest that memory B cells are essential for the induction and persistence of SLE, and that effective SLE therapies might require specific depletion or suppression of

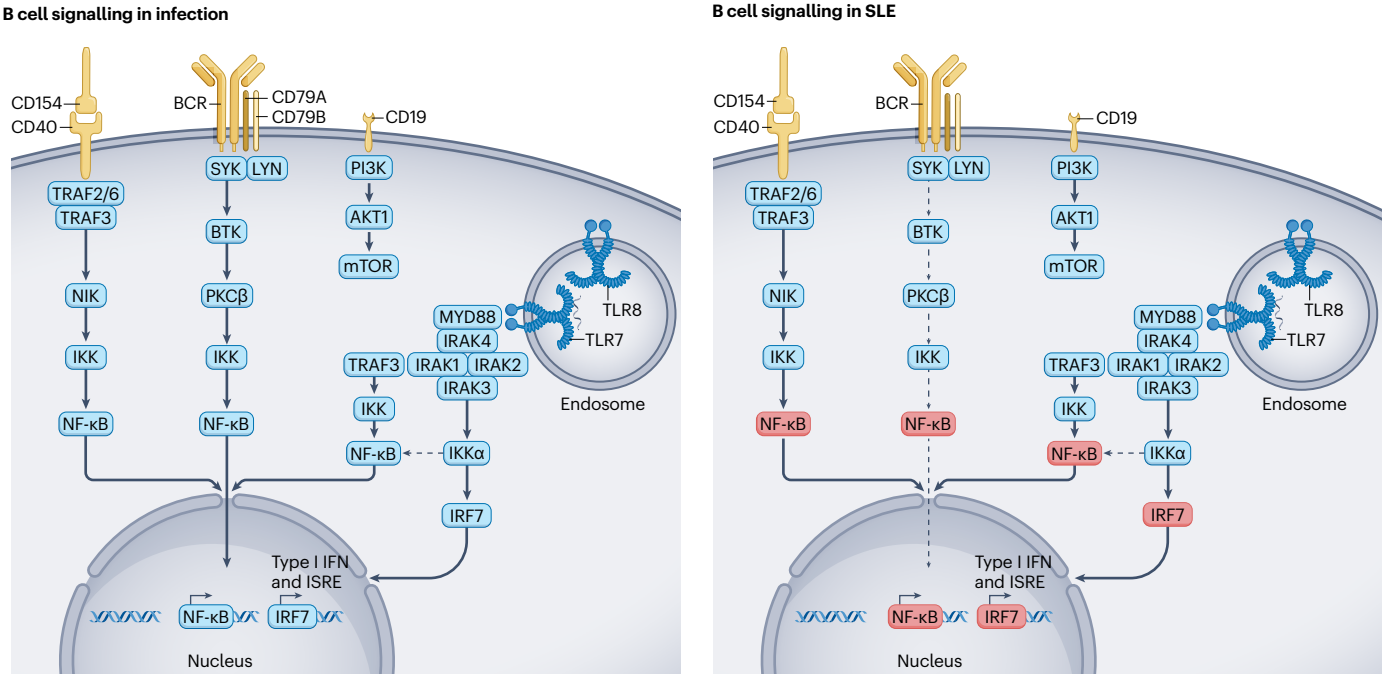

Fig. 1 | Signalling abnormalities in B cells, especially memory B cells, in systemic lupus erythematosus. a, In B cells from healthy individuals, stimulation of the B cell receptor (BCR) induces phosphorylation of the downstream signalling cascade via the kinases SYK, LYN and BTK, finally resulting in adequate activation of transcription factor NF-κB. In addition, CD19 signalling leads to the activation of the PI3K and AKT and the downstream initiation of mTOR signalling. Ligation of CD40 by CD154 (also known as CD40L) leads to engagement of TNFR-associated factors (TRAFs) and the downstream activation of NF-κB-inducing kinase (NIK) and IκB kinase (IKK), leading to the activation of NF-κB. In addition, the binding of endosomal single-stranded RNA molecules to Toll-like receptor 7 (TLR7) or TLR8 leads to engagement of the adaptor molecule MYD88 and downstream recruitment of IL-1 receptor-associated kinases (IRAKs), leading to the downstream activation of NF-κB and interferon regulatory factor 7 (IRF7). **b**, In patients with systemic lupus erythematosus (SLE), memory B cells have a hyporesponsive BCR with inadequate phosphorylation of the BCR signalling cascade but accessible TLR7 and TLR8 signalling initiated within endosomes and activated by single-stranded RNAs, as well as a functional CD40 activation pathway activated by the CD154 molecule on T helper cells. IFN, interferon; ISRE, IFN-stimulated response element.

this B cell population. We argue that current B cell-directed therapies are only active insofar as they affect the memory B cell population and that B cell-directed therapies will only achieve improved and persistent efficacy if they ensure eradication or re-education of this B cell population. Moreover, we suggest that targeting other B cell subsets, such as plasma cells, might confer some transient benefit by the reduction of autoreactive plasma cells fuelling the formation of pathogenic immune complexes, but disease recrudescence is inevitable if SLE-associated memory B cells persist and reinitiate disease immunopathogenesis.

In this Review, we highlight preconditions and early events leading to the emergence of autoreactive B cells and SLE initiation, as well as the ensuing mechanisms of memory B cell formation and reactivation, and discuss how abnormalities in memory B cell responses, including BCR hyporesponsiveness combined with sustained TLR signalling and activation via bystander T cells might explain the continuous breach of peripheral tolerance, the preferential autoantibody repertoire, and female sex bias in SLE. We next propose that a positive feedforward loop involving both innate (TLR7 or TLR8) and adaptive (CD40) immune signalling underlies the memory B cell reactivation and autoreactive plasma cell differentiation in SLE, and discuss how disruption of this feedback loop might correlate with efficacy of B cell-targeted therapeutic strategies.

SLE predisposition and pathogenic B cells

Sex bias and other genetic factors

Several factors known to predispose to SLE, such as female sex or certain HLA class II haplotypes, appear to be important contributors to abnormal B cell function in this disease. In particular, SLE displays a striking female sex bias, with approximately 90% of the people affected being women[24]. Various hypotheses, including the hormone–hormone receptor signalling hypothesis, have been proposed to explain this phenomenon. A compelling explanation of the female sex bias in SLE relates to gene dosage of X chromosome-encoded immune molecules. Notably, molecules with a key role in innate and adaptive immune activation are encoded on the X chromosome, including TLR7, TLR8, interleukin-receptor associated kinase-1 (IRAK1), Bruton's tyrosine kinase (BTK), CD154, as well as X-inactive specific transcript (Xist) (Supplementary Fig. 1). Incomplete inactivation of X chromosomal loci in women might result in a gene dose-dependent increase in relevant immune functions promoting female sex bias for type I interferon-associated rheumatic autoimmune inflammatory diseases (RAIDs)[25]. The same mechanisms might also confer enhanced protection against virus infections in women, which might explain their lower morbidity and mortality[26,27] following infections. Thus, improved virus protection appears to be associated with an enhanced risk of autoimmunity in females, and this

risk seems to be at least in part related to certain immune molecules encoded on the X chromosome.

Accordingly, early associations of systemic autoimmunity with TLR7 duplications[28] and incompletely silenced X chromosome genes in Klinefelter syndrome (XXY)[29] were followed by recognition of the association of the X chromosome-encoded long non-coding RNA Xist with the female sex bias of RAIDs[30]. Xist encodes a long non-coding RNA that is universally expressed and regulates X chromosome inactivation[31]. Immune genes that escape X chromosomal silencing[28], such as *TLR7* (refs. 32,33), *CD154*, *CXCR3*, *BTK*, *TASL*, interleukin-2 receptor (*IL2R*) or *IRAK1*, have been associated with the expansion of atypical memory B cells in humans with autoimmunity and in mouse models of autoimmunity and ageing. In addition, interaction of Xist with numerous RNA-binding proteins[30] appears to increase the immunogenicity of the latter and has also been associated with the expansion of atypical memory B cells and ABCs[30].

Another important genetic precondition of adaptive immunity is related to HLA class II haplotypes. The DR2 alleles DRB1*1501/DQB1*0602 and the DR3 alleles DRB1*0301/DQB1*0201 were found to be present in nearly two-thirds of 780 patients with SLE and their family members. DR2 haplotypes have been associated with autoantibodies to the nuclear antigen Sm, whereas DR3 genotypes have been associated with SSA/Ro-specific and SSB/La-specific autoantibodies. Thus, HLA class II DR2 and DR3 haplotypes are key elements involved in specific autoantibody production and susceptibility to SLE[34].

Early emergence of autoantibodies

In SLE, current data clearly support the view that abnormal B cell activation and the associated breach of immune tolerance give rise to autoantibodies years before SLE manifestations[35,36]. There is considerable evidence that T cells are involved in establishing the autoimmune B cell and plasma cell repertoire in SLE, resulting in the emergence of typical autoantibodies years before autoimmune disease onset (Supplementary Fig. 2). In individuals producing typical autoantibodies without overt disease, increased levels of type II interferon (IFNγ) are an early abnormality[36], and these findings combined with the known contribution of IFNγ to T cell responses suggest that initial production of autoantibodies is largely controlled by T cells[7,34] (Supplementary Fig. 2). Upon occurrence, most anti-ribonucleoprotein (anti-RNP) antibodies are strongly associated with the type I interferon signature[37], but the plethora of SLE-related autoantibodies makes it very difficult to understand the role of each of them in driving disease. With the exception of anti-Sm antibodies, other anti-RNP autoantibodies do not occur exclusively in SLE. Thus, an early break of immune tolerance precedes the onset of SLE, but the induction mechanisms of autoimmunity in these individuals remain largely to be delineated.

B cell abnormalities in SLE initiation

Skewed B cell repertoires have been noted in SLE[19,38], suggesting abnormalities in both B cell differentiation within germinal centres or at extrafollicular sites and B cell survival within lymphoid organs and peripheral tissues. Resulting perturbances in B cell function as a result of chronic BCR cell stimulation or innate immune cell-derived and T cell-derived cytokines have provided important new insights and clearly support a role for B cells, and especially for memory B cells, in SLE pathogenesis.

T cell help

Although there are essential requirements for T cell collaboration in the induction of immunoglobulin class switching and somatic hypermutation of immunoglobulin heavy chain genes, the detailed contribution of T cells to B cell responses during established SLE remains to be resolved. Patients with SLE have reduced numbers of regulatory T cells (T_{reg} cells) and increased numbers of T helper 17 cells (T_H17 cells), associated with an imbalance between IL-2 and IL-17 levels[25]. In addition, tissue-resident memory T cells (T_{RM} cells) that produce the chemokine CXCL13 and are able to attract B cells into germinal centres and potentially other tissues have been implicated in SLE pathogenesis[8]. $CXCL13^+$ T_{PH} and T_{FH} cells appear to be expanded and related to persistent type I IFN-driven T cell abnormalities. Even though these cells may be important in stimulating B cell responses, the effectiveness of B cell-directed therapies suggests they have no other essential functions in disease pathogenesis[39–41].

Perturbed naive B cell function and repertoire

A $CD19^+CD20^+CD5^+CD38^+CD10^+CD9^+IgD^+CD27^-$ population of pre-naive B cells that are enriched in autoantibody specificities has been shown to be expanded in SLE[42]. As with other early B cell subsets, this population responds suboptimally to BCR activation, but can be activated via CD40 engagement[42]. Importantly, the lack of complete culling of autoreactivity in this pre-naive B cell population suggests that, when activated by bystander help, or by TLR7 engagement, pre-naive B cells are likely to differentiate into memory B cells and plasma cells or plasmablasts with enrichment in autoantibody specificities[43].

Conventional $CD27^-$ naive B cells from patients with SLE also respond suboptimally to BCR engagement[42]. This phenotype resembles the BCR anergic status of B cells seen in chronic viral infections[44], suggesting that chronic in vivo activation, particularly when combined with prolonged exposure to type I interferon, might induce partial anergy already in the premature immune repertoire. Indeed, elevated STAT1 expression in B cells and T cells from patients with SLE is a typical sign of long-term exposure to type I interferon signalling[45]. This persistent type I interferon environment might also be an important factor for maintaining BCR hyporesponsiveness[22].

In summary, naive B cells with a hyporesponsive BCR occur in patients with SLE, potentially as a result of chronic exposure to autoantigens, type I interferons or both. The observations of naive B cell repletion and reduced type I interferon expression in responders following B cell depletion are consistent with the assumption that type I interferon contributes to the abnormal BCR responsiveness of naive B cells in SLE[44]. Interestingly, signalling via the largely intact CD40 (T cell) and TLR activation pathways can overcome the anergic status of conventional $CD27^-$ B cells from patients with SLE. Thus, a unique set of abnormalities can skew the ability of both pre-naive and naive B cells to be properly activated and regulated in SLE[22]. Suboptimal BCR signalling might also underlie the compromised immune protection against infections in SLE. Whether any of these abnormalities can be targeted uniquely by novel therapeutics is currently not known, although the possibility that reducing the exposure to type I interferon and/or engagement by TLR7 and TLR8 ligands offer potential approaches for future evaluations.

Abnormal memory B cell function

Functionally, memory B cells from healthy individuals have greatly reduced requirements to differentiate into plasma cells compared with naive B cells. This characteristic is important for rapid immune protection against pathogens and for mounting adequate vaccine responses. Memory B cells rapidly differentiate into plasma cells in response to IL-21 and BAFF signalling, even in the absence of T cells

or BCR engagement, and this rapid response ensures survival from infection[46,47].

In RAIDs, there is considerable evidence of the pathogenic relevance of the memory B cell compartment. Initially, phenotypic abnormalities of peripheral B cell subsets in SLE[38,48] and other RAIDs were identified, with expanded conventional memory B cells, atypical DN1, DN2 and DN3 memory B cells or CD11c$^+$ ABC subsets, and with peripheral plasmacytosis[18,49]. Functional memory B cell abnormalities have been identified in both conventional CD27$^+$IgD$^+$ and atypical CD27$^-$IgD$^-$ B cells[17,22] (Fig. 1), along with a unique dependence on glycolysis for both survival and function[50]. These findings make it plausible that memory B cells are biologically different in patients with SLE than in healthy controls, and function as independent drivers of disease (Box 1).

In SLE, conventional and atypical memory B cells can be extensively reactivated outside germinal centres, especially within extrafollicular sites and autoimmune tissues[8,18,51]. Light zone-like structures containing FDCs are usually not detectable in extrafollicular sites and in tissues affected by autoimmune activity, indicating that peripheral negative selection of autoreactive clones might be defective in extrafollicular or in situ autoimmune responses (Fig. 2). In this context, autoreactive clones can emerge in SLE and thereby differ from conventional memory B cell reactivation as observed for secondary vaccine responses (Box 1). In summary, the reactivation of memory B cells and induction of the atypical memory B cell subsets DN1, DN2 and DN3 in SLE are largely confined to extrafollicular and in situ tissue activation contributing to the critical expansion of the memory compartment and insufficient mechanisms to control autoreactivity.

The disbalance between anergic BCR and responsive TLR7 signalling in anti-RNP$^+$ memory B cells in SLE might also contribute to the selective expansion of memory B cells outside the germinal centres. There is clear evidence of memory B cell activation at extrafollicular sites[38,52,53], such as proliferative lymphoid nodules (PLNs) within the spleen, which, in the case of immune thrombocytopenia[51], are distant from germinal centres or in some circumstances nearby atrophic germinal centres, or within affected tissues[17], such as the tubulointerstitial tissue in lupus nephritis[54]. PLNs within the spleen or kidney lack important features of germinal centres, including the polarized FDCs, and maintain autoantigen presentation even during steady state. As such, reactivation of memory B cells in the absence of the regulatory influences of FDCs might perpetuate ongoing autoimmune responses. Of particular note, T_{PH} cells have been associated with the expansion of DN1, DN2, DN3, and CD11c$^+$ ABC atypical memory B cells within autoimmune tissues[1,8]. However, it still remains to be clarified whether T_{PH} cells simply co-localize with B cells within affected tissues or also provide help to these B cells.

Box 1 | Immune activation during established autoimmune disease does not follow principles of conventional secondary activation

Spatial abnormalities: In systemic lupus erythematosus (SLE), memory B cell reactivation is largely confined to extrafollicular and autoimmune tissue reactivation lacking germinal centre regulation and negative selection. During secondary vaccine challenges, memory B cell reactivation occurs preferentially in secondary lymphoid organs with germinal centres and at extrafollicular sites.

Functional abnormalities: In SLE, memory B cells have an anergic phenotype, with B cell receptor (BCR) hyporesponsiveness as a result of increased protein tyrosine phosphatase activity that controls BCR signalling. Toll-like receptor (TLR) signalling and CD40 signalling remain intact. TLR7 and TLR8 signalling overrides the response to BCR activation, and autoantibody specificities are fixed as B cells do not undergo affinity maturation. Autoantibodies are, thus, often specific to TLR ligands, and although they are usually mutated and of high avidity, maturation of BCR binding during the disease does not occur. Moreover, cytokine (mainly type I interferon) signalling is constitutive, lacking sequential adaptation of the immune response via cytokine switch. The emerging autoreactive memory B cell and plasma cell compartments are not contracting over time. By contrast, after vaccination, BCR affinity is finely tuned, and orchestrated cytokine sequences impact on immunoglobulin switch and somatic hypermutation to increase the immunoglobulin repertoire exposed to negative selection by follicular dendritic cells in the light zones of the germinal centres. Subsequently, the memory compartments become quiescent and contract.

Metabolic abnormalities: In SLE, B cells predominantly undergo glycolysis, and this is not observed in control B cells.

Converging pathogenic pathways

Type I interferon and B cell abnormalities co-occur in patients with SLE[8,45] or some other RAIDs, including in patients with extraglandular Sjögren syndrome[55] and a subset of patients with rheumatoid arthritis (RA)[56], which suggests that these two types of dysregulation might be interrelated during both the initiation and the maintenance of autoimmune disease (crossroad hypothesis).

More specifically, emerging evidence indicates that perturbed memory B cell activity resulting in the production of anti-RNP autoantibodies and the upregulation of the type I interferon pathway converge to potentiate autoimmune disease[43]. From a reverse translational perspective, the downregulation of type I interferon signalling upon selective B cell depletion treatments and the association of disease recurrence with memory B cell repletion are supportive of this model (for details see below). Memory B cells appear to be the major focal point of the convergence with dysregulation of the type 1 interferon pathway (Fig. 2). Although the rudiments of memory B cell generation are similar in autoimmunity and in response to exogenous antigens, some aspects are distinct (Box 1). In particular, protective responses result in orchestrated downregulation of immune activation, including the downregulation of type I interferon signalling, when the infection is resolved[44]. In individuals predisposed to autoimmunity, this resolution of immune activation leads to chronic autoimmunity and a continuous immune stimulation that potentially overrides peripheral negative selection of autoreactive B cell clones[6,17]. The resulting memory B cells have autoreactive specificities and a dysregulated signalling programme manifested as BCR hyporesponsiveness and enhanced responsiveness to bystander T cell help and TLR7 and TLR8 engagement. As a result, these memory B cells can respond to T_{PH} cells at extrafollicular foci or in autoimmune tissues and differentiate into autoantibody-secreting plasmablasts or plasma cells that produce autoantibodies, including anti-RNP autoantibodies. Notably, they are not exposed to censoring by FDCs[4].

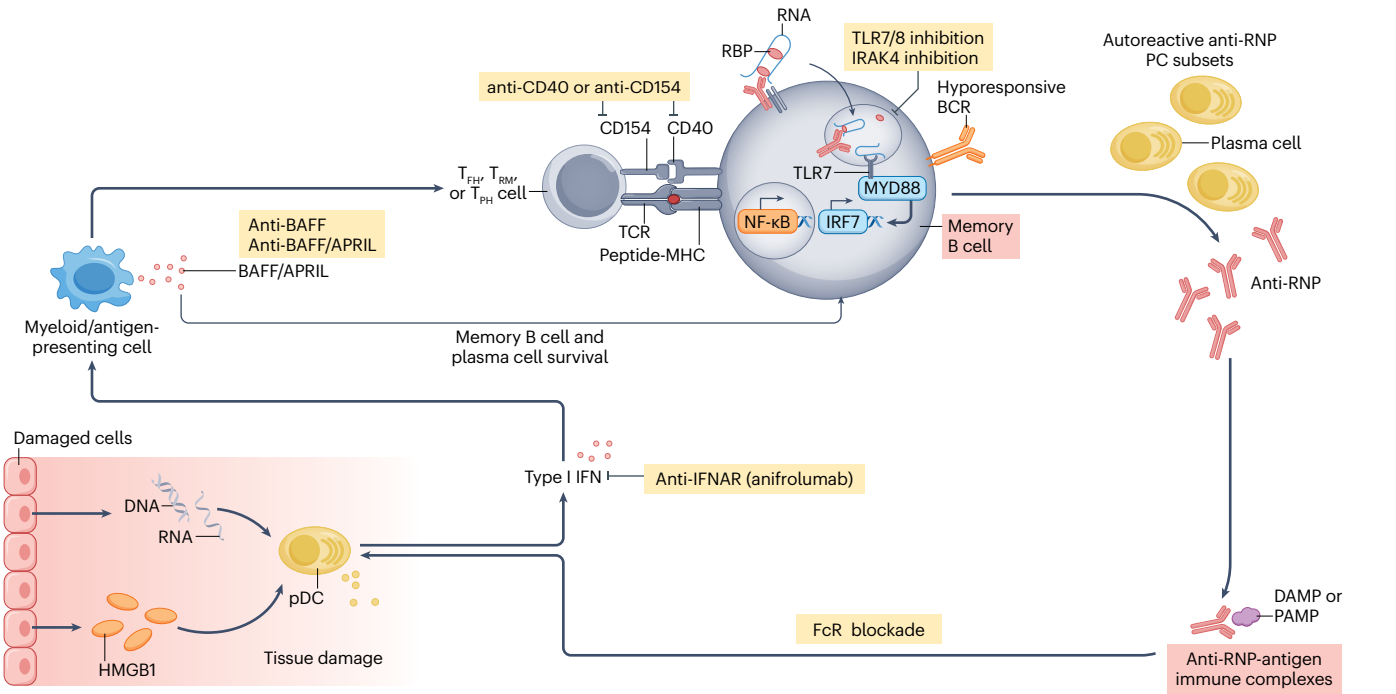

Fig. 2 | A positive feedforward loop links abnormal memory B cell signalling, autoantibody production and the type I interferon signature of SLE. During initiation of systemic lupus erythematosus (SLE), naive B cells and their corresponding autoreactive plasma cells are primed to recognize ribonucleoprotein (RNP) autoantigens at germinal centres years before disease onset. Genetic factors such as certain HLA class II haplotypes predispose towards the activation of B cells that recognize RNP antigens, leading to the generation of autoreactive memory B cells and $CD19^{-}$ long-lived plasma cells that reside in the bone marrow, where they produce anti-RNP autoantibodies. Following disease initiation, B cell receptor (BCR) signalling is anergic or dysfunctional and the BCR can only function to internalize the cognate autoantigens. Following antigen internalization, memory B cells are reactivated via Toll-like receptor 7 (TLR7) signalling, and this process defines the spectrum of autoantibodies with largely TLR7 specificities. Notably, memory B cell reactivation occurs outside germinal centres with support from T peripheral helper (T_{PH}) cells or tissue-resident memory T (T_{RM}) cells which reside within areas that do not support effective peripheral negative selection, such as the extrafollicular areas of lymphoid tissues or the autoimmunity-affected tissues, where follicular dendritic cells (FDCs) are absent. The resulting autoantibodies form immune complexes that fuel the activation of plasmacytoid dendritic cells (pDCs), which produce type I interferons (IFN) and support the production of BAFF by myeloid cells. The feedforward loop comprises a unique but crucial interaction between the two cellular subsets, memory B cells (probably including the atypical memory B cell subsets DN1, DN2 and DN3) and autoreactive plasma cells as well as the two key cytokines type I interferon and BAFF. These elements along with TLR7 and TLR8 ligands and CD40 bystander stimulation define the destiny of anti-RNP memory B cells and are all important drivers of SLE pathology. Non-depleting strategies such as anti-CD40 or anti-CD154 monoclonal antibodies, TLR7 and TLR8 inhibitors, interleukin-receptor associated kinase 1 (IRAK4) inhibitors, blockade of Fc receptors (FcR), interferon α receptor (IFNAR)-specific monoclonal antibody anifrolumab, or anti-BAFF and anti-APRIL monoclonal antibodies, that are able to interfere with the proposed model are indicated within yellow fields. DAMP, damage-associated molecular pattern; PAMP, pathogen-associated molecular pattern; PC, plasma cell; RBP, RNA-binding protein; TCR, T cell receptor; T_{FH}, T follicular helper.

Anti-RNP antibodies

Anti-RNP autoantibodies build immune complexes with RNA binding proteins[57], and these immune complexes can feedback on memory B cells via engagement of TLR7 and TLR8. In addition, the immune complexes along with type I interferon itself enhance production of BAFF, further propagating memory B cell stimulation[58]. This evolving pattern of events, with memory B cells acting at the crossroad, might account for the propagation of autoimmunity and provides many targets for intervention. Even when the hyporesponsive BCR (Fig. 1) does not transmit an appropriate intracellular activation signal, we hypothesize that it might function to internalize autoantigens[22], including the TLR7 and TLR8 ligands bound to anti-RNP immune complexes. Based on their characteristics, the BCR might identify certain RNP structures, whereas TLR7 and TLR8 signalling is activated strictly by nucleic acids. The crossroad hypothesis, as such, might also provide a clue concerning how intracellular and intranuclear autoantigens drive autoantibody production even when their localization excludes their direct binding by cognate autoantibodies. This concept might explain why anti-RNP autoantibodies dominate across diseases with distinct clinical phenotypes that only share a common type I interferon signature (SLE, Sjögren syndrome, RA subsets, mixed connective tissue disease).

A positive feedforward loop for B cell activation

After development of autoantibodies in individuals without overt disease who subsequently develop SLE, an increase of type II interferon has been noted[36], consistent with the hypothesis that T cell involvement potentially instructs B cells at this stage. Subsequently, and shortly before disease presentation, the levels of type I interferon were found to increase, indicating a potential contribution of type I interferon during the stage immediately preceding the onset of clinical manifestations[36].

These findings suggest that the pathway towards clinical disease involves several steps: initial B cell activation is followed by effector T cell activation and, next, by an innate immune response that heralds the onset of clinical manifestations.

During established SLE, we propose that a positive pro-inflammatory feedforward loop (Fig. 2), interconnects abnormal memory B cell reactivation with increased type I interferon and BAFF levels as well as with the high titres of anti-RNP autoantibodies that are produced by plasma cells and form immune complexes, which both stimulate memory B cells and activate plasmacytoid dendritic cells to produce more type I interferon. The observation of decreased type I interferon signatures after selective B cell depletion or reduced autoantibody levels[39,59] is consistent with such a unique feedforward loop connecting the B cell and plasma cell compartments.

Collectively, there is clear evidence of a central role of memory B cells and their associated autoreactive plasma cells in SLE pathogenesis. The two B cell compartments are crucial elements in the positive feedforward loop leading to chronic production of type I interferon not observed in protective immunity.

Implications for B cell therapies

Translational insights about the impact of B cell therapies on autoantibody production, immune complex formation and type I interferon production have substantiated the crucial role of memory B cells in RAIDs. B cell-targeting therapies have permitted such insights (Fig. 3). In support of our proposed model that incorporates suboptimal BCR signalling, the targeting of molecules involved in BCR-mediated activation (BTK inhibitors (BTKi)[60,61], anti-CD22 (ref. 62) and anti-CD19 combined with Fc receptor inhibition[63]) has failed in SLE trials. By contrast, B cell depletion strategies (anti-CD20 (ref. 64), CD19-targeting chimeric antigen receptor (CAR) T cells[39], anti-CD52 (ref. 65) and autologous stem cell transplantation (ASCT)[66]) that can each deplete memory B cells with variable efficiencies have led to varying degrees of naive B cell repopulation and reduced autoantibody levels, as well as decreased type I interferon expression (Fig. 3).

Insights from B cell cytokine targeting

Belimumab is an approved monoclonal antibody that blocks BAFF, a cytokine of the TNF superfamily[67] Belimumab impacts atypical memory B cells by diminishing bystander help required for their activation[68]. Interestingly, ABCs express high levels of the BAFF receptor (BAFFR)[7] and their numbers are increased in SLE. Treatment with belimumab resulted in remarkably reduced numbers of atypical memory B cells[69], decreased expression of activation markers by DN B cells[70] and diminished autoantibody production[71,72]. Moreover, contraction of two memory B cell clusters – surface IgA-positive memory B cells, which are likely to be activated within extrafollicular sites or tissues, and $CD11c^{+}CD21^{-}$ ABCs[68] – was associated with improved responses to belimumab. Belimumab has also been noted to affect certain plasma cell subsets and reduce IgM and, to a lesser extent, IgG and IgA production[72]. An initial increase in peripheral memory B cells is well documented for the first weeks of treatment with belimumab[69] followed by a subsequent decline in memory B cell numbers below baseline. In addition, patients treated with belimumab develop deactivated, non-proliferative recirculating memory B cells with features of disrupted lymphocyte trafficking[73], possibly representing displaced tissue resident memory B cells. Finally, patients treated with belimumab for up to 312 weeks (6 years) had remarkable decreases in all B cell subsets[74]. Thus, belimumab not only interferes with survival of certain antibody-producing cells resulting in reduced autoantibody titres and immune complex formation, but also impairs the differentiation of atypical memory B cells and causes displacement of tissue-resident memory B cells. In further support of our model, a meta-analysis of all registered belimumab trials found improved responses to belimumab in patients who had elevated baseline levels of BAFF protein or medium-to-high *BAFF* and type I *IFN* mRNA levels[75]. These findings further support a relationship between BAFF and type I interferon levels in SLE.

TACI (TNFRSF13B) Fc fusion proteins that block both BAFF and APRIL (also known as TNFsf13), including atacicept[76] and telitacicept[77], have been studied in SLE. BAFF and APRIL both provide survival signals to plasma cells but their differential impact on certain plasma cell subsets remains to be delineated. Telitacicept showed remarkable efficacy in a phase II clinical trial in SLE[77] and is currently undergoing further development for potential registration. An important signature of both TACI Fc fusion proteins is their impact on all immunoglobulin isotypes produced by plasma cells (IgM and IgG, but also IgA). Importantly, the differential impact of BAFF and APRIL on memory B cell generation and differentiation into plasma cells has not been fully studied, although BAFF has been shown to have an important role in this process[24]. How APRIL and BAFF distinctly impact serum IgM, IgA and IgG levels requires further delineation. In this context, selective anti-APRIL blockade by the monoclonal antibody sibeprenlimab has been tested in IgA nephropathy and has provided additional evidence that APRIL is important for IgA production. A phase II study showed a dose-dependent effect on proteinuria[78] in this otherwise difficult to treat nephropathy. In IgA nephropathy, autoantibodies target the galactose-deficient hinge region of IgA1, which leads to the formation of pathogenic immune complexes[79]. Sibeprenlimab treatment decreased the levels of all IgA, including of galactose-deficient IgA1. The role of IgA autoantibodies in SLE remains uncertain but circulating IgA^{+} plasmablasts, probably of mucosal origin, are found to be increased in SLE[80]. Therefore, studies of selective APRIL blockade might hold value in SLE.

Insights from direct B cell targeting

The dysfunctional signalling status of memory B cells in SLE with the anergic BCR but retained TLR and CD40 responsiveness is a central element of the proposed positive feedforward loop and appears to be supported by the outcomes of strategies blocking BCR signalling by targeting downstream kinases, such as BTK or SYK. Lack of efficacy in SLE has been noted for the BTKi fenebrutinib[61] and evobrutinib[60] (Fig. 3), supporting the hypothesis that BCR signalling is defective in memory B cells in SLE and that its inhibition is unlikely to result in clinical benefit[12]. In addition, the lack of clinical benefit of the non-depleting CD22-blocking antibody epratuzumab that also interferes with BCR signalling[81] is consistent with the above hypothesis[22,82]. Further, the non-cytolytic monoclonal antibody obexelimab[63] that binds CD19, which is part of the BCR complex, and Fcγ receptor IIb (FcγRIIb) and is able to inhibit shared signalling pathways downstream of the BCR in B cells also did not show efficacy in a phase II trial. The aggregate of these data supports the view that BCR signalling is unnecessary in chronic SLE and that targeting BCR signalling is unlikely to be successful in SLE. By contrast, BTKi molecules have shown efficacy in relapsing–remitting multiple sclerosis[83] and Sjögren syndrome[84], although inhibition of BCR signalling has shown limited value in RA[85]. The differences between overall B cell depletion responses and responses to BTKi represent unique opportunities to disentangle subtle functional B cell abnormalities in the various autoimmune and inflammatory diseases.

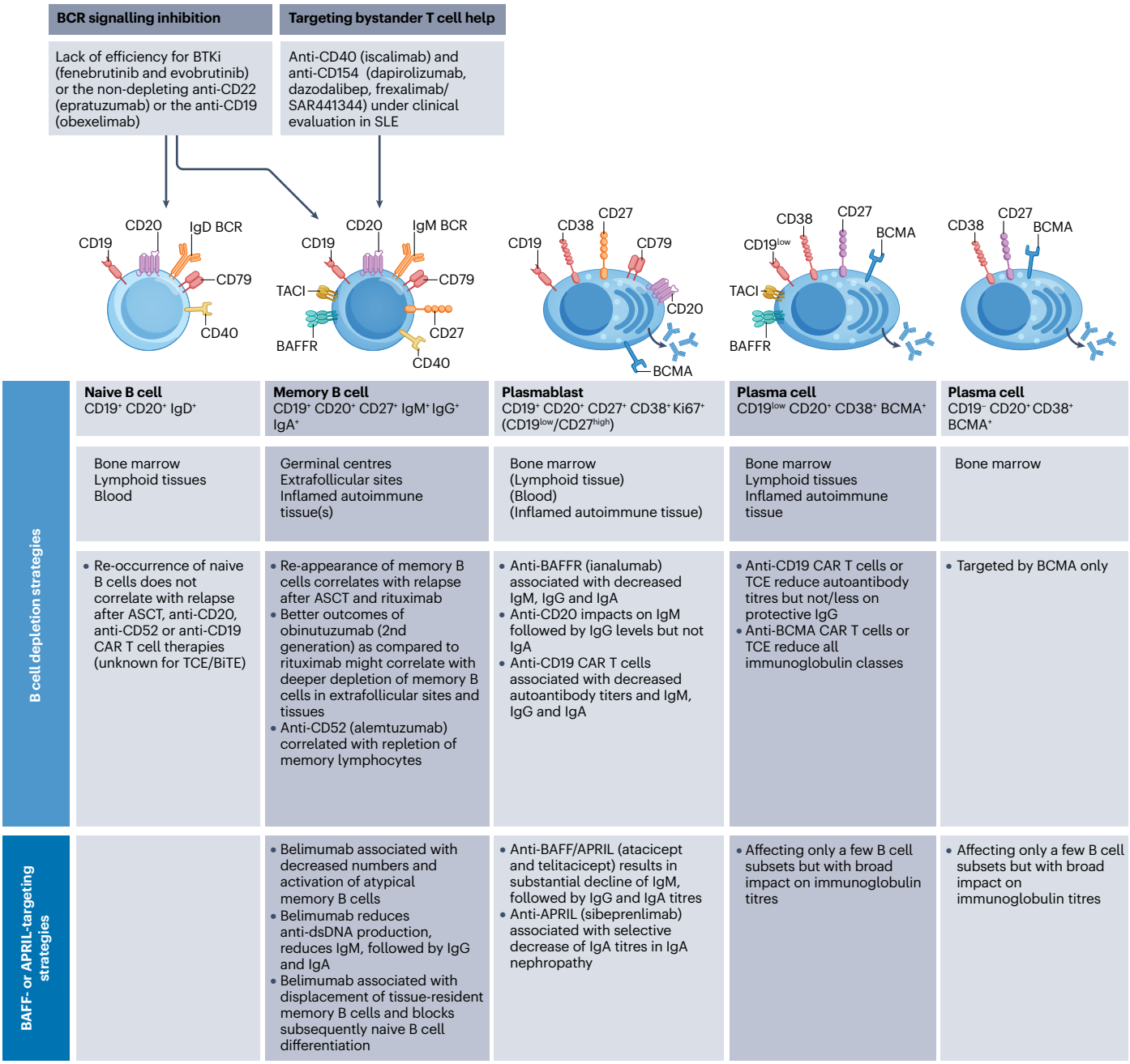

Fig. 3 | Selective targets of B cell depletion. The development of B cells is accompanied by changes in surface molecule expression, some of which have been applied in clinical interventions. Anti-CD20, anti-CD19, anti-CD38 and anti-BAFF receptor (BAFFR) targeting results in differential B cell depletion including $CD19^{low}$ plasma cells (which preferentially reside in the bone marrow). A notable distinction between CD19 and CD20 targeting relates to the broader coverage of CD19 targeting from pro B cells through $CD19^{low}$ bone marrow plasma cells. It remains to be delineated how deep depletion of tissue-resident B cells, including atypical double-negative memory B cell subsets as well as germinal centre-resident B cells in lymphoid and target tissues can be achieved. Here, potential differences between anti-CD20 and anti-CD19 (chimeric antigen receptor (CAR) T cells with lymphodepletion/bispecific T cell engagers (BiTEs)) might provide advantages. CD19 targeting is also considered to differentially affect the bone marrow plasma cell compartment, leaving only $CD19^-$ bone marrow plasma cells untouched and remaining unaffected by CD20 targeting. Targeting BAFF or BAFFR as well as APRIL (by a direct antibody) or targeting of BAFF and APRIL by atacicept and telitacicept, respectively, are expected to differentially impact on certain bone marrow plasma cell subsets. In this context, anti-CD38 as well as anti-BCMA targeting is considered to completely deplete bone marrow plasma cells. The aggregate of these different depletion possibilities of B cell lineage cells will not only provide new treatments for individual patients but also very detailed insights into B cell lineage development in health and autoimmunity. Note: During development, CD38 expression extends until the pre-naive stage. ASCT, autologous stem cell transplantation; BCR, B cell receptor; BTKi, Bruton's tyrosine kinase inhibitors; dsDNA, double-stranded DNA; SLE, systemic lupus erythematosus; TCE, T cell engager.

Box 2 | Potential approaches to target autoreactive plasma cells

Several innovative approaches have been proposed to target autoreactive plasma cells and thereby reduce the source of high autoantibody levels that otherwise result from abnormal memory B cell activation in systemic lupus erythematosus (SLE). Such approaches mostly involve the selective inhibition of plasma cell differentiation or plasma cell survival and are listed below.

1. Targeting of plasma cell differentiation via the blockade of the transcription factors IRF4, PRDM1 or XBP1 (ref. 109).
2. Proteasome inhibition to interfere with protein turnover and, thereby, affect plasma cell survival. Bortezomib has been used to inhibit overall proteasome activity[110], whereas zetomipzomib has been used for the selective inhibition of the immunoproteasome[111]. Zetomipzomib acts very selectively only on certain subunits of the immunoproteasome complex and is currently under clinical development for the treatment of lupus nephritis.
3. Plasma cell depletion with anti-CD38 (ref. 59) monoclonal antibodies[92,112].
4. Targeting of BCMA on the cell surface of plasma cells. BCMA has been effectively targeted in a mouse model of SLE[50], and a bispecific BCMA-targeted and CD19-targeted chimeric antigen receptor (CAR) T cell therapy is currently being tested in a phase I clinical trial[89]. In addition, an anti-BCMA and anti-CD3 bispecific antibody or with the BCMA-targeted bispecific T cell engager (BiTE) teclistamab have shown good responses in patients with SLE[40]. As BiTEs are off-the-shelf therapeutics, they might hold advantages over CAR T cell therapeutics for clinical applications, as they circumvent the need and risks of lymphodepletion and the challenges of preparing a genetically engineered cellular therapeutic. BCMA-targeted CAR T cells, which were initially developed to eradicate myeloma clones in patients with multiple myeloma, have shown promising responses in SLE[89] and other autoimmune diseases (neuromyelitis optica spectrum disorder[113], anti-SRP necrotizing myopathy[114,115] and myasthenia gravis).

Another approach to interfere with B cell activation and differentiation is modulation of T cell–B cell interaction by targeting checkpoint molecules. This is usually a non-depleting strategy and targets intracellular signalling pathways that are distinct from BCR signalling but provide co-stimulation for proper activation of naive B cells[4]. Here the CD40–CD154 pathway is crucial for T cell-dependent B cell activation and is required for many events in germinal centre reactions (B cell differentiation, immunoglobulin class switching, somatic hypermutation) and the reactivation and proliferation of established memory B cells at extrafollicular sites and within tissues. Importantly, CD40 signalling is intact in memory B cells in SLE and might mediate bystander T cell help to activate memory B cells in an antigen-independent manner. Blockade of CD154 by a humanized monoclonal antibody (BG9588, 5c8) has been shown to normalize peripheral B cell abnormalities in lupus nephritis[86]. Currently, monoclonal antibodies targeting CD40 (iscalimab and others) or CD154 (dapirolizumab, dazodalibep and frexalimab/SAR441344)[87] are in clinical development. A central aim of targeting checkpoint molecules such as CD40 in SLE would be to prevent bystander T cell help from rescuing memory B cells from BCR hyporesponsiveness and promoting memory B cell reactivation.

Years of experience with depleting anti-CD20 therapies (rituximab, ocrelizumab and ofatumumab) with regulatory approval for various indications, and the concept of depleting a broad spectrum of B cell lineage cells from pre-B cell to memory B cells (Fig. 3) have provided evidence that rejuvenation of the B cell system is feasible with sufficient efficacy and safety[11,64]. Safety was a key concern during the initial phase of anti-CD20 therapy development[88]. Subsequently, strategies were developed to target either a broader range of B cell subsets (CD19-targeted[39] or CD19 and BCMA co-targeted CAR T cells[89] and bispecific T cell engagers (BiTEs)[39,90,91]) or selective plasma cell populations (therapies targeting BCMA or CD38 (ref. 92)) (Box 2). The underlying studies will provide unprecedented insights into the distinct contributions of B cell subsets including the autoantibody-producing plasma cell subset in individual patients. A prediction based on the feedforward model is that strategies that deplete or reprogramme memory B cells might be necessary to obtain long-term remission, whereas those that decrease plasma cell numbers and autoantibody titres might be associated with transient or incomplete responses because of the continuous tick-over of memory B cells to autoantibody-producing plasma cells.

Evidence obtained from studies of B cell lineage depletion as a result of ASCT has implications regarding the requirement for extensive reduction of extrafollicular and tissue-resident memory B cells ('deep depletion') in SLE[66,93,94]. For example, following ASCT, an intervention that affects the entire adaptive immune system but has been associated with remission for over 5 years in about 70% of patients with SLE[95], the reappearance of naive T cells and B cells was not associated with disease recurrence[41,96]. Instead, it was the emergence of memory T cells and B cells that was linked with relapse. Similarly, following B cell and T cell co-targeting with anti-CD52 (alemtuzumab[65]), disease recurrence was related to memory lymphocyte repletion, although success was limited by overall toxicity owing to long-term lymphopenia and neutropenia with increased infection risk[97]. Collectively, strategies simultaneously depleting T cells and B cells have been associated with substantial risks of infections and secondary autoimmunity[97,98] but have also provided support to the hypothesis linking a lasting clinical response to the extensive depletion of memory B cells.

The introduction of selective B cell depletion strategies permitted additional mechanistic insights. However, anti-CD20 strategies with rituximab or ocrelizumab were only marginally effective in SLE. The reasons are manifold but might in part relate to incomplete depletion of tissue-resident memory B cells or extrafollicular B cells. In this context, second-generation anti-CD20 (obinutuzumab) with enhanced antibody-dependent cellular cytotoxicity (ADCC) and binding to a different CD20 epitope showed clinical efficacy in lupus nephritis and an association with peripheral B cell depletion[99]. An alternative explanation of limited anti-CD20 activity was the insufficient targeting of memory T cells, in particular T_{RM} cells in target tissues. In this regard, incomplete depletion of memory B cells might require that the T cells driving their tick-over to autoantibody-producing plasma cells are fully depleted to achieve clinical benefit. New strategies with the potential to achieve complete memory B cell depletion might obviate the requirement for T cell depletion. One main finding in long-term anti-CD20 therapies of SLE was that sustained clinical responses were noted when the re-populating B cells were dominated by naive B cells[100].

Alternative B cell depletion strategies comprise anti-BAFFR targeting with ianalumab[101], a monoclonal antibody with the capacity to both block BAFF binding and deplete BAFFR-positive cells, resulting in more

effective tissue depletion of B cells and partially targeting bone marrow plasma cells (BMPCs). Notably, the expression of BAFFR is higher on atypical memory B cells and on some plasmablasts or plasma cells than on other B cell subsets[7]. Reduction of all immunoglobulin isotypes (IgM, IgG and in particular IgA) with ianalumab treatment[102] could be a surrogate marker indicating that the treatment impacts at least certain BMPCs that are not depleted with anti-CD20 treatment.

Recently, CD19-based B cell targeting has been the subject of regained interest. Despite the limited effectiveness of the anti-CD19 monoclonal antibody in SLE[103], recent experience with CD19-targeted CAR T cells[39] has shown remarkable responses in patients with autoimmunity, including eight patients with SLE who achieved DORIS remission without continuous immunosuppressive treatment. Following treatment with CD19-targeting CAR T cells, patients achieved long-term remission, although B cell depletion lasted only 112 ± 47 days[39]. The correlation between naive B cell dominance after treatment and clinical response is consistent with observations in patients responding to ASCT[41]. These data further validate the finding that memory B cells are key drivers of RAIDs and also link memory B cell depletion with a diminished type I interferon signature, which is consistent with the proposed feedforward loop hypothesis. CD19-targeted CAR T cells also selectively decreased autoantibody versus protective antibody titres, confirming that the two distinct plasma cell populations ($CD19^-$ and $CD19^{low}$ cells)[104] might be clinically relevant. The data imply that deeper tissue depletion of B cells by migrating CAR T cells might account for the higher efficacy compared with anti-CD20 antibody treatments. With regard to targeting BMPCs, bispecific CD19-targeted and BCMA-targeted CAR T cells also showed promising efficacy and tolerability in 13 patients with SLE[89]. This strategy is able to completely deplete BMPCs, in contrast to CD19 targeting, which is considered to deplete only a $CD19^{low}$ BMPC subfraction[104]. The differential role of the conditioning regimen, appropriate CAR T targets and optimal patient populations remain to be delineated when compared with monoclonal antibody therapies.

The aggregate of currently available data suggests that depletion of the entire memory B cell compartment is key to success in treating SLE. The key learning may be the association between clinical response and successful depletion of the memory B cell compartment. Importantly, naive B cells are apparently less important in disease pathogenesis, as active disease does not occur when naive B cells return. To what extent memory B cell depletion is a precondition for blocking differentiation of plasma cells and autoantibody production remains to be determined in further studies.

Strategies targeting innate signalling pathways

In order to interfere with the consequences of the feedforward feedback loop that is reinforced by the autoantibody-initiated immune complexes and type I interferon signalling, selective blockade of TLR7 and TLR8 activation[105] or IRAK-4 inhibition[106] represent innovative interventions to block these innate immune activation pathways (Fig. 2), and are being assessed in early clinical studies. It will be of interest to study whether blockade of these pathways results in clinical efficacy and how the resulting data shed new or confirmatory light on the SLE model outlined above. As most of the anergic lymphocytes in SLE undergo metabolic adaptations with increased glycolysis, (semi)selective metabolic approaches by itaconate, metformin or 2-deoxy-D-glucose might also show potential in reverting abnormal B cell activation or status, although no clinical studies have yet been announced.

Although non-depleting interventions might be supported by a mechanistic rationale and by experiences with belimumab and anifrolumab, responses to these interventions require time. Most intriguing and timely responses were found to B cell depletion strategies that result in persistent memory B cell depletion and subsequent repopulation by naive B cells.

Limitations and future research directions

Considering the complexity of innate and adaptive immune signals that converge at memory B cells according to the crossroad hypothesis,

Glossary

Age-associated B cells
(ABCs). B cells that increase in number as a result of ageing, viral infections, immunodeficiency and autoimmune diseases (rheumatoid arthritis and systemic lupus erythematosus). ABCs are identified by CD11c expression.

Atypical memory B cells
A term largely applied to $CD27^-IgD^-$ B cells that lack expression of CD27, a marker of memory B cells, but otherwise have features of B cell memory.

Follicular dendritic cells
(FDCs). Cells of mesenchymal origin that are found in the germinal centre light zone of primary and secondary lymphoid tissue. FDCs capture and present antigens to support B cell activation and, along with CD40–CD40L-based B cell–T cell interactions, ensure negative selection of autoimmune B cells.

Germinal centres
Transiently formed structures within the B cell zone (follicles) in secondary lymphoid organs that harbour a dark zone where immunoglobulin class switching and somatic hypermutation are taking place and a light zone where BCR/immunoglobulin selection occurs based on T cell and follicular dendritic cell interactions.

Heavy and light chains of the B cell receptor
Antibody molecules are composed of two immunoglobulin heavy chains and two immunoglobulin light chain proteins, the variable regions of which define their binding specificity.

T follicular helper cells
(T_{FH} cells). T_{FH} cells are antigen-experienced $CD4^+$ T cells expressing PD1 and typically producing IL-21, able to initiate and maintain germinal centre formation within secondary lymphoid organs.

T peripheral helper cells
(T_{PH} cells). Unlike T follicular helper cells (T_{FH} cells), which interact with B cells within lymphoid organs, T_{PH} cells provide help to B cells, and especially to memory B cells, within inflamed tissues, supporting plasma cell differentiation. Distinct features of T_{PH} cells, as compared with T_{FH} cells, are the expression of CXCR5, which is associated with T_{PH} cell localization within inflamed tissues, and a low BCL6 to BLIMP1 ratio. T_{PH} cells depend on various cytokines for their survival within tissues, such as IL-6, type I interferon and IL-12 or IL-23.

Tissue-resident memory T cells
(T_{RM} cells). $CD4^+$ memory T cells that express BCL6 and are crucially involved in the development of autoimmune B cell and $CD8^+$ T cell memory responses. T_{RM} cells can permit the activation of B cells at extrafollicular or tissue sites and thus escape censoring by germinal centres.

TLR7 and TLR8
Members of the Toll-like receptor family and innate receptors DAMPs (damage-associated molecular pattern molecules) able to recognize GU-rich single-stranded RNA (ssRNA) (TLR7) or U-rich ssRNA (TLR8) in endosomes and to initiate B cell activation in the contexts of viral and autoimmune responses.

questions arise about the crucial role of T cell–B cell interactions in SLE beyond the impact of CD40–CD154. The extent to which continuous activation of T cells, including T_{RM} cells, in affected tissues might contribute[8] to the proposed positive feedforward loop remains unclear. Therefore, the impact of memory T_{PH} and T_{FH} cell subsets requires further research, especially given that they appear to resist selected CD20 and CD19 depletion.

Memory B cell activation might be differentially fuelled by signalling downstream of TLR7 versus CD40 across individual patients with SLE, and biomarker profiling might inform treatment selection, favouring either TLR7 and TLR8 inhibition or CD40–CD40L blockade[107,108]. Patient stratification will be of utmost importance given that various treatments become available in the clinic.

Finally, clinical research in SLE permits unique opportunities for translational and reverse translational insights into immunology. Based on the initial identification of antinuclear autoantibodies as a diagnostic marker, we have entered a period of a better understanding of the underlying mechanisms and developing targeted therapies. In this context, selective memory B cell depletion in SLE is currently not feasible because of the lack of distinct surface markers, but future research may identify such opportunities.

Conclusions

An aggregate of data from basic, translational and clinical research emphasizes the crucial role of memory B cells in SLE, a disease that involves pathogenetic pathways at the cellular crossroads of innate and adaptive immunity. The preferential expansion of autoreactive memory B cells with suboptimal BCR signalling but intact responsiveness to TLR ligands or bystander T cells engaging CD40 appears to explain several SLE characteristics. First, this hypothesis suggests that the female predominance in SLE might be associated with the incomplete silencing of the X chromosomal genes *TLR7* or *CD40L*. Second, the impairment of protective immune responses and associated susceptibility to infections in patients with SLE appears to be independent of medication but related to the decreased BCR responsiveness. Third, the perpetuated production of anti-RNP antibodies by autoreactive plasma cells might be explained based on the hyporesponsive BCR and accessible TLR7 and CD40 signalling as combined with the preferential internalization and processing of cognate TLR7 ligands (RNPs) and the expansion of bystander T cells. The key aberrations in memory B cell biology might also explain the ineffectiveness of certain strategies inhibiting BCR signalling pathways in SLE. Nevertheless, the aggregate of ongoing studies applying various depleting and non-depleting B cell interventions will provide unprecedented insights into mechanisms of autoimmunity and will also contribute to basic knowledge of humoral immunity while potentially identifying novel targets for effective treatment.

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