REVIEWS

Cytokines in the pathogenesis of rheumatoid arthritis

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Abstract | Cytokines regulate a broad range of inflammatory processes that are implicated in the pathogenesis of rheumatoid arthritis. In rheumatoid joints, it is well known that an imbalance between pro- and anti-inflammatory cytokine activities favours the induction of autoimmunity, chronic inflammation and thereby joint damage. However, it remains less clear how cytokines are organized within a hierarchical regulatory network, and therefore which cytokines may be the best targets for clinical intervention a priori. Here, we discuss the crucial effector function of cytokines in the immunological processes that are central to the pathogenesis of rheumatoid arthritis.

Citrullinated peptide
A peptide that incorporates the amino-acid citrulline. This amino acid is generated post-translationally by peptidylarginine deiminases. Patients with rheumatoid arthritis generate characteristic autoantibodies against the part of the antigenic determinant that contains citrulline moieties.

Shared epitope
A common stretch of amino acids in the peptide-binding grooves at positions 67–74 of the HLA-DR β-chain.

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Rheumatoid arthritis is a chronic inflammatory disease that mainly targets the synovial membrane, cartilage and bone. It affects 1% of the population and is associated with significant morbidity and increased mortality[1]. Cytokines are directly implicated in many of the immune processes that are associated with the pathogenesis of rheumatoid arthritis. Numerous cytokines are expressed and are functionally active in synovial tissues. Accordingly, cytokine modulation alters the outcome in many rodent models of arthritis. Importantly, tumour-necrosis factor (TNF) is now targeted in the standard treatment of patients with rheumatoid arthritis. Other cytokines are being tested as targets in the clinic, with promising results[2,3]. Such proof of concept in human disease provides important validation of the pre-clinical models that have led to the elucidation of putative roles for cytokines in the pathology of this disease and others. Here, we discuss how cytokines are involved in the pathogenesis of rheumatoid arthritis. The roles of cytokines within a complex regulatory network are related to specific immunological processes that promote autoimmunity, chronic inflammation and tissue destruction. We propose that elucidating such networks and defining the functional hierarchies therein can present novel opportunities for controlling the disease and ultimately for the induction of disease remission.

Aetiopathogenesis of rheumatoid arthritis?

Unifying hypotheses for the cause of rheumatoid arthritis must explain several key features of the disease, namely autoimmunity, chronic inflammation and joint destruction (FIG. 1). Autoimmunity, which manifests as the production of antibodies specific for IgG (known as rheumatoid factors) or specific for cyclic citrullinated peptides, precedes the clinically detectable onset of inflammatory arthritis[1] and can sometimes last for years (and therefore can be referred to as the pre-articular phase). The early mechanisms by which T-cell and B-cell tolerance are breached are poorly defined, but probably arise at a systemic immune regulatory level, in aberrant thymic selection or peripheral tolerance. Nevertheless, set on this autoimmune-prone background, some subsequent event triggers articular localization, the onset of inflammatory synovitis and therefore clinical presentation. The crucial triggers for the onset of articular disease are unknown but they probably include biomechanical factors, neuroimmunological interactions and altered articular microvascular function. Several genetic loci have been proposed to have an association with the susceptibility and severity of rheumatoid arthritis. A disease association with HLA-DR4 alleles (which contain the shared epitope) is well established. Other loci, including *PTPN22* (protein tyrosine phosphatase, non-receptor type 22), *PADI4* (peptidyl arginine deiminase, type IV), *CTLA4* (cytotoxic T-lymphocyte antigen 4), FcγRs (Fc receptors for IgG), and various cytokine and cytokine-receptor loci, such as those encoding TNF, interleukin-1 (IL-1), IL-10 and IL-18, have been implicated in disease association to various degrees in distinct populations (reviewed in REF. 4). Environmental factors also have an effect on the induction, magnitude and rate of progression of the disease. Although numerous infectious organisms have been implicated, recent data most strongly implicate smoking as an important environmental risk factor for the development of disease in HLA-DR4-positive individuals[5]. Given that smoking

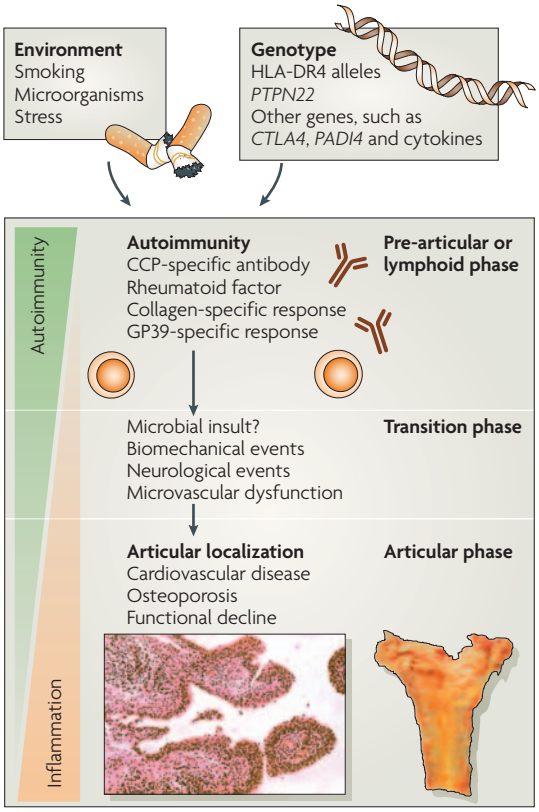

Figure 1 | **A contextual framework for the pathogenesis of rheumatoid arthritis.** Autoimmune processes are predicted to occur up to years prior to the clinical onset of disease and represent a pre-articular or lymphoid phase of disease. Transition to the articular phase, which corresponds to the clinical manifestation of the disease, is initiated by ill-defined processes, such as biomechanical and neurological events. Thereafter inflammation-driven pathogenesis occurs, which leads to joint destruction and increases co-morbidity, including cardiovascular disease and osteoporosis. Autoimmune processes may defer to inflammatory pathways as the disease progresses. CCP, cyclic citrullinated peptide; *CTLA4*, cytotoxic T-lymphocyte antigen 4; GP39, cartilage glycoprotein 39; *PADI4*, peptidyl arginine deiminase, type IV; *PTPN22*, protein tyrosine phosphatase, non-receptor type 22.

promotes the citrullination of self proteins, it is possible that such environmental influences might be directly linked to pathogenic autoantigen-driven responses.

After onset of clinical disease, the normally hypocellular synovial membrane becomes hyperplastic, comprising a superficial lining layer of synovial fibroblasts and macrophages, overlying an interstitial zone that contains a marked cellular infiltrate, which includes synovial fibroblasts, macrophages, mast cells, $CD4^+$ T cells, $CD8^+$ T cells, natural killer (NK) cells, NKT cells, B cells and plasma cells. The inflamed synovium invades adjacent cartilage and promotes articular destruction, which is mediated by the activities of osteoclasts, chondrocytes and synovial fibroblasts. The underlying bone marrow also exhibits an inflammatory infiltrate, containing T-cell–B-cell aggregates, and so the bone probably receives a bidirectional insult[6]. Articular damage in turn probably generates a rich source of neo-antigens to promote further autoimmune reactivity. In addition, the articular environment is profoundly hypoxic and angiogenesis is a characteristic feature of rheumatoid joints[2,3]. Rheumatoid joints therefore consist of an unusual pathophysiological environment comprising hypoxia and variable biomechanical stress that may render *in vivo* immune signalling pathways distinct from those described in optimized model systems *ex vivo*.

Cytokines are implicated in each phase of the pathogenesis of rheumatoid arthritis, by promoting autoimmunity (including during the pre-articular phase, at least in animal models of arthritis), by maintaining chronic inflammatory synovitis and by driving the destruction of adjacent joint tissue. Cytokines therefore integrate the immune-regulatory and tissue-destructive events that underlie the clinical presentation and progression of rheumatoid arthritis.

In this Review, we focus largely on the roles of cytokines in the various clinical phases of rheumatoid arthritis in relation to the involved tissues, namely the joints. However, some of the features of this disease may, in part, be explained by the systemic activities of cytokines released from the inflamed synovium, from secondary lymphoid organs, or indeed from target tissues. For example, IL-6 directly regulates the release of the acute-phase proteins from hepatocytes and Kuppffer cells (reviewed in REF. 7). TNF promotes cachexia, depressed mood and altered cognitive function. Several pro-inflammatory cytokines probably drive the accelerated vascular disease that has been described in patients with rheumatoid arthritis and contribute directly to altered function of adipocytes (including adipocytokine release), lipid metabolism, insulin resistance and acquired metabolic syndrome[8].

T-cell function in rheumatoid arthritis

T cells are implicated in the pathogenesis of rheumatoid arthritis by virtue of the genetic association with MHC class II alleles and with the lymphoid-specific *PTPN22*, the detection of high numbers of T cells in the inflamed synovium and the demonstrated requirement of T cells in various animal models of arthritis[9]. However, therapeutic approaches based on modulating T cells, such as with cyclosporin, CD4-specific antibody and CD52-specific monoclonal antibody (alemtuzumab; Campath-1H, Genzyme Corporation and Schering AG) have been disappointing[10]. However, some therapeutic benefit has been seen in patients treated with the fusion protein CTLA4–immunoglobulin Fc, which supports a role for T-cell co-stimulation and effector T-cell activation in rheumatoid arthritis[11].

Synovial T-cell function and differentiation. Cytokines regulate the phenotype of effector and regulatory T cells in the synovium. The main cytokine growth factor for synovial T cells is IL-15, but this does not directly modulate their cytokine effector phenotype[12]. Largely on the basis of studies of rodent models, rheumatoid arthritis has been considered a T helper 1 (T_H1)-cell-mediated disorder, and therefore was thought to be driven by a population of T cells producing inflammatory cytokines and

Osteoclasts
Multinucleated giant cells, of the monocyte lineage, that are responsible for bone resorption. Osteoclasts degrade bone matrix and solubilize calcium from bone. Problems with their differentiation and a decrease in their number lead to bone osteopetrosis. Conversely, an increase in their number or function induces bone osteoporosis, indicating that osteoclasts have a pivotal role in bone homeostasis.

Acute-phase proteins
A group of proteins, including C-reactive protein, serum amyloid A, fibrinogen and α1-acid glycoprotein, that are secreted into the blood in increased or decreased quantities by hepatocytes in response to trauma, inflammation or disease. These proteins can be inhibitors or mediators of inflammatory processes.

Cachexia
Severe weight loss, muscle wasting and debility caused by prolonged disease. It is thought to be mediated through neuroimmunoendocrine interactions.

Cyclosporin
A commonly used immunosuppressive drug that blocks calcineurin A and thereby inhibits T-cell activation. It is used to prevent the rejection of transplanted organs and to treat some inflammatory diseases.

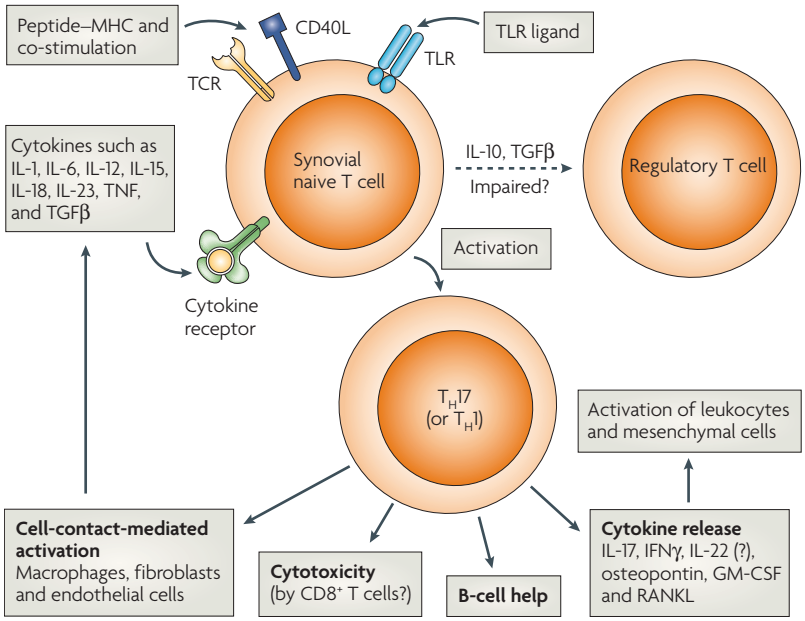

Figure 2 | **Pathways leading to activation of synovial T cells in rheumatoid arthritis and their key effector pathways.** Synovial T cells may be activated by T-cell receptor (TCR) and co-stimulation pathways and by cytokine- or Toll-like receptor (TLR)-driven stimuli. In particular, the synovial milieu contains interleukin-12 (IL-12), IL-23, IL-6 and transforming growth factor-β (TGFβ), and as such promotes the differentiation of T helper 1 (T_H1) and T_H17 cells. Regulatory T cells, although present, may not exhibit optimal regulatory activity. In rodent models, regulatory T cells are present in high numbers in the joints, whereas in human disease the relative contribution of these subsets remains unknown. Activated T cells mediate effector function in rheumatoid arthritis through the release of cytokines, to promote downstream leukocyte and mesenchymal-cell activation, through the provision of help to B cells and, in the case of $CD8^+$ effector T cells, cytotoxic activity. They also activate macrophages, fibroblasts and endothelial cells through direct cell contact. CD40L, CD40 ligand; GM-CSF, granulocyte/macrophage colony-stimulating factor; RANKL, receptor activator of nuclear factor-κB (RANK) ligand; IFNγ, interferon-γ; TNF, tumour-necrosis factor.

Collagen-induced arthritis
(CIA). An animal model of rheumatoid arthritis. CIA develops in susceptible rodents and primates after immunization with cartilage-derived type II collagen.

T-bet
A member of the T-box family of transcription factors. It is a master switch in the development of T helper 1 (T_H1)-cell responses, through its ability to regulate expression of the interleukin-12 receptor, inhibit signals that promote T_H2-cell development and promote the production of interferon-γ.

ELISPOT
A method based on antibody capture for enumerating specific T cells ($CD4^+$ and $CD8^+$) that secrete a particular cytokine (often interferon-γ).

chemokines, such as interferon-γ (IFNγ), lymphotoxin-β (LTβ) and TNF[13]. However, more recently, studies in animal models favour a new model that implicates IL-17-producing T cells (a recently characterized subpopulation of T cells known as T_H17 cells) as crucial effectors[14]. This model is supported by the observation of accelerated collagen-induced arthritis (CIA) in mice that lack the T_H1-cell-associated genes *Ifng*, *Ifngr* or *Il12p35* (REFS 15–17), and although mice deficient in the T_H1-cell-associated transcription factor T-bet develop less severe CIA, this is attributed to altered dendritic cell (DC) function, owing to a reduced secretion of IL-1β and inflammatory chemokines[18]. By contrast, and consistent with a role for T_H17 cells in this disease, *Il6*$^{-/-}$ mice and *Il23p19*$^{-/-}$ mice are resistant to the development of CIA[17,19], and inhibition of IL-17 or overexpression of IL-17 in the joints suppresses or worsens joint inflammation and damage, respectively[14]. Moreover, the induction of T-cell self-reactivity in SKG mice — a strain of mouse that spontaneously develops inflammatory arthritis — depends on the production of IL-6 by antigen-presenting cells and T cells and on the generation of T_H17 cells, whereas IFNγ deficiency exacerbates disease[20]. These data are consistent with the hypothesis that T_H17 cells, which can be induced by IL-6, transforming growth factor-β (TGFβ) and IL-23, act as the key effector-cell subset in inflammatory arthritis, at least in rodents.

Whether these mouse models faithfully represent human disease is currently unclear. Nevertheless, consistent with the T_H17-cell model, IFNγ is lacking, or present at low levels, in the synovial membrane of patients with rheumatoid arthritis and it is rarely detectable in the synovial fluid[21], although ELISPOT analyses and phenotyping studies of synovial T-cell clones support the presence of some T_H1 cells in rheumatoid joints[22,23]; such cells may only transiently produce T_H1-type cytokines *in situ* or exist at a low frequency. IL-17 expression by perivascular T cells, which presumably represent T_H17 cells, has been detected in rheumatoid synovial membranes[24]. However, further studies are required to confirm these data, as is the investigation of other IL-17-family members, particularly IL-17F.

The precise site of differentiation of T cells into pathogenic effector T cells in rheumatoid arthritis is not known. The synovial milieu, at least in established disease, contains various macrophage- and synovial-fibroblast-derived cytokines, such as IL-1β, IL-6, IL-7, IL-12, IL-15, IL-18, IL-23p19 and TGFβ, that can support the expansion and differentiation of T_H1 cells and/or T_H17 cells (FIG. 2). Myeloid and plasmacytoid DC subsets might also contribute to T-cell differentiation in the synovium through the production of IL-12p70, IL-23p19, IL-15 and IL-18 (S. L. Jongbloed and M. C. Lebre, unpublished observations). Whether other IL-12-family cytokines have an inflammatory role in rheumatoid arthritis is unclear. The observation of a reduction in adjuvant-induced arthritis by the administration of IL-27-specific antiserum[25] is not consistent with evidence from other models of autoimmune disease, such as experimental autoimmune encephalomyelitis, which indicate a net negative regulatory role for IL-27 in disease[26,27]. That reciprocal regulation between T_H1 and T_H17 cells has been reported in murine models[28], and may occur in the synovium of patients with rheumatoid arthritis, might complicate the interpretation of these studies. One further proviso is that the stage of disease probably alters the cytokine milieu in the joint[29]. For example, synovial lavage of individuals with very early rheumatoid arthritis revealed elevated levels of IL-4 and IL-13, whereas the synovial fluid of patients with established disease is characterized by a lack of IL-4 and consistently low levels of IL-13 (REF. 29).

Naturally occurring T_{Reg} cells (that is, the forkhead box P3 (FOXP3)$^+$CD4$^+$CD25$^+$ regulatory T-cell subset) have been detected in the synovium of patients with active disease and particularly in synovial fluid, but they seem to have impaired regulatory function[30,31]. The cytokine factors that sustain the expansion of T_{Reg} cells in the rheumatoid joint are not defined but probably include the regulatory cytokines IL-10 and TGFβ. A recent study of the effect of TNF-blocking antibodies indicates that TNF-dependent pathways might also modulate the regulatory capacity of T cells. Inhibition of TNF in patients with rheumatoid arthritis was shown to promote the emergence of a FOXP3$^+$CD4$^+$CD25$^+$CD62L$^-$

regulatory T-cell population that could suppress effector T cells through TGFβ- and IL-10-dependent pathways[32]. This could in part explain the beneficial effects of this therapeutic approach.

The requirement for local antigen in driving specific T-cell populations in synovitis is debated[33]. T-cell receptor (TCR) oligoclonality and evidence of B-cell somatic hypermutation in the joints clearly indicate that specific antigen-driven events do occur[34]. Evidence for self-reactive T cells, such as against citrullinated peptides, type II collagen and cartilage glycoprotein 39 (GP39), has also been provided. However, the cytokine milieu significantly modulates TCR-dependent responses, as exemplified by the uncoupling of the TCR ζ-chain signalling by chronic exposure to TNF[35], and the net contribution of self-reactivity in the chronic phase of the disease is poorly defined. It is likely that distinct pathogenic pathways could operate in early disease or in a putative state of immune dysregulation that coincides with a pre-articular phase of rheumatoid arthritis. Elucidation of such specificities and T-cell effector function would in turn reveal optimal pathways to tolerance induction that are ultimately necessary to achieve long-term, drug-free clinical remission of disease.

Effector pathways whereby T cells promote synovitis. Synovial T cells could contribute to synovitis directly through the production of inflammatory cytokines (FIG. 2). Commensurate with the above discussion, the role of IFNγ as an effector cytokine in joint inflammation is unclear. Although addition of IFNγ to cultures of synovial cells activates various effector pathways, including cytokine production by macrophages, and collagen synthesis and cytokine release by synovial fibroblasts, the *in vivo* significance of this pathway is unclear, as the levels of IFNγ in the synovium are low. Moreover, the administration of IFNγ to patients with rheumatoid arthritis did not significantly modulate disease[36]. Considerable interest therefore lies in the effector function of T-cell-derived IL-17. IL-17 drives neutrophil differentiation, maturation, activation and cytokine release; monocyte activation and cytokine release; and synovial-fibroblast activation, cytokine and chemokine release, prostaglandin production and matrix metalloproteinase (MMP) synthesis[28]. The activation of DCs in the joint by IL-17 together with TNF is also likely. A synergistic effect has also been observed with low concentrations of IL-17, IL-1β and TNF, which together leads to synovial-fibroblast activation and cytokine production, indicating a pathogenic role for these inflammatory cascades[37]. A potent role for IL-17 in joint damage has also been proposed[14,38] (as discussed later).

That T_H17 cells may mediate their effects via other cytokines is also now becoming clear. For example, the IL-10-family member IL-22 is also produced by T_H17 cells in response to IL-6 or IL-23 stimulation and has recently been shown to promote inflammation in the skin and to modulate cutaneous acanthosis[39]. The expression of IL-22 and its receptor have been detected in rheumatoid synovial membranes, but rather than being associated with T cells they were mainly associated respectively with $CD68^+$ or $vimentin^+$ cells, which are indicative of macrophages or synovial fibroblasts[40]. This may reflect the presence of TGFβ in the synovium, as TGFβ opposes IL-22 production by T_H17 cells. TGFβ therefore offers ambivalent inflammatory effects in synovitis: together with IL-6, it can promote the differentiation of cells into T_H17 cells, but in the relative absence of IL-6 (such as after therapeutic blockade of the IL-6 receptor), it can favour the induction of T cells with a regulatory phenotype[28]. Other T-cell-derived cytokines may also be found to have a role. Osteopontin, for example, is an extracellular matrix protein that has cytokine-like properties by binding to several integrins and CD44. Interestingly, osteopontin-deficient mice exhibit reduced disease in arthritis models[41], and osteopontin expression is detected in the synovial membrane and synovial $CD4^+$ T cells of individuals with rheumatoid arthritis, where it seems to act as a paracrine and autocrine amplification factor for cytokine release in the joints[42], in particular inducing the production of IL-1 and various chemokines.

T cells also contribute to synovial inflammation via direct interactions with neighbouring macrophages and synovial fibroblasts that promote their activation. Accordingly, freshly isolated synovial T cells induce TNF and IL-1β release from syngeneic macrophages in a cell-contact-dependent manner[12]. Moreover, culturing peripheral blood $CD4^+$ or $CD8^+$ T cells with combinations of IL-2, IL-6, IL-15 or TNF recapitulates their macrophage-activating properties *in vitro*[12,43]. Such cytokine-activated T cells closely resemble those found in the synovium with regard to their effector profile and the signal cascade that they induce in macrophages[43], which indicates that this may be an important pathway for sustaining cytokine release *in vivo*. Importantly, the activation status (that is, by TCR ligation or by cytokine activation) and phenotype (T_H1 or T_H2) of the T cell determines what signalling pathways (such as through phosphoinositide 3-kinase (PI3K) versus nuclear factor-κB (NF-κB) activation) are triggered in target macrophages, and what cytokines and chemokines are released by target macrophages[43,44]. The crucial receptor–ligand pairs implicated in the T-cell–macrophage interactions are unresolved, but include CD40–CD40L (CD40 ligand), LFA1–ICAM1 (lymphocyte function-associated antigen 1–intercellular adhesion molecule 1), CD2–LFA3 and CD69 (REFS 12,44,45). T-cell–macrophage interactions can be suppressed by IL-4, IL-10 and TGFβ, and by apolipoprotein A1 in paracrine regulatory loops[44]. Synovial fibroblasts are also activated by cognate interactions with activated T cells, mainly through T-cell presentation of membrane-bound cytokines to synovial fibroblasts, indicating that this may be a general effector pathway in chronic inflammation.

Regulation of B cells in synovitis

The advent of B-cell-depleting therapeutics, such as the CD20-specific monoclonal antibody rituximab (Rituxan; Genentech, Inc. and Biogen Idec Inc.), in rheumatoid arthritis has refocused interest in pathways that regulate B-cell activation, maturation and function in the joints. Rituximab depletes all B-cell subsets, except plasma cells (which lack CD20 expression), and induces significant

Plasmacytoid DC
A dendritic cell (DC) subset with a morphology that resembles that of a plasmablast. Plasmacytoid DCs produce large amounts of type I interferons in response to viral infection.

Adjuvant-induced arthritis
An experimental animal model of arthritis, in which disease is induced by the administration of heat-killed mycobacteria in oil.

and sometimes long-lived clinical benefit[46]. In addition to autoantibody production, and thereby immune-complex formation, the B-cell lineage contributes to pathogenesis by the production of cytokines and chemokines (such as IL-6, IL-10 and LTβ). B-cell-derived cytokines regulate the activation of follicular DCs and lymphoid neogenesis, and contribute regulatory feedback loops for T-cell–macrophage and T-cell–B-cell interactions. However, further studies are required to properly characterize the cytokine synthetic capacity of B cells in the synovium. Their role as antigen-presenting cells may also be important in the joints, as B-cell depletion prevents the formation of ectopic germinal centres and the optimal activation of T cells[47].

B-cell survival and activation signals are mainly provided by TNF-superfamily members. Mature ($CD83^+$) DCs in synovial membranes that contain ectopic germinal centres produce high levels of the B-cell survival factor APRIL (a proliferation-inducing ligand; also known as TNFSF13)[48], and macrophages and synovial fibroblasts in most synovial tissues can produce BAFF (B-cell-activating factor; also known as BLYS and TNFSF13b)[49]. The exposure of synovial fibroblasts to TNF and IFNγ in co-culture was sufficient to induce high levels of BAFF production and support B-cell survival and activation. Disruption of the BAFF–BAFF-receptor interaction with the TACI (transmembrane activator and calcium-modulating cyclophilin-ligand interactor)–immunoglobulin Fc fusion protein inhibits antibody production and cytokine expression by B cells in the human-synovium–SCID mouse model[48]. However, so far, therapeutic targeting of BAFF in patients with rheumatoid arthritis has been unsuccessful. Resolving the contribution of the cytokine milieu to B-cell survival and effector function will therefore be essential to optimize future therapeutic manipulation.

The lymphocyte infiltrate in the synovium comprises various patterns of structural organization: cells can be diffusely distributed, loosely aggregated or form ordered structures that contain germinal centres (occurring in <20% of individuals affected with rheumatoid arthritis). The formation of ectopic germinal centres may contribute to the improper regulation of emerging self-reactive B cells, as a result of local affinity maturation and receptor editing[50]; their presence may predispose to poorer outcome. Cytokines and chemokines are directly implicated in this lymphocyte organization. CXC-chemokine ligand 13 (CXCL13) and CC-chemokine ligand 21 (CCL21) promote the formation of synovial germinal centres[51]. Accordingly, CXCL13 and CCL21 expression is found at lower levels in the tissues with loosely aggregated lymphocytes[52]. Germinal-centre formation in the synovium also requires the expression of LTβ, at least by B cells[53]; a role for LTα in germinal-centre formation has been implicated but does not seem to be essential. The membrane-bound heterotrimeric form of lymphotoxin, $LT\alpha_1\beta_2$, promotes the release of cytokines (IL-1, IL-6 and granulocyte/macrophage colony-stimulating factor (GM-CSF)), chemokines (CCL2 and CCL5) and matrix metalloproteinases (MMP1 and MMP3) by synovial fibroblasts, which express the LTβ receptor (LTβR)[54]. In turn, all of these synovial-fibroblast-derived factors may support the recruitment and activation of T cells. Synovial fibroblasts have been attributed 'follicular-DC-like' activity in some[55], but not all[54], studies. Finally, an important and unexpected role for synovial $CD8^+$ T cells in ectopic germinal-centre organization has been implicated by cell-specific depletion studies in the human-synovium–SCID mouse model[56]. Synovial $CD8^+$ T cells accumulated in the mantle zone of germinal centres, and expressed IFNγ and CD40L, but not perforin. Precisely how these $CD8^+$ T cells are involved in germinal-centre formation is unclear but it may be through the expression of $LT\alpha_1\beta_2$.

The pathophysiological and clinical importance of such synovial micro-architecture remains to be determined and will require long-term follow-up studies, in particular to determine the effect on these structures of therapeutic cytokine inhibition.

Effector leukocyte function in synovitis

Macrophages. Macrophages are considered an important source of synovial pro-inflammatory cytokines. Activation of and subsequent cytokine production by macrophages (and synovial fibroblasts) in the synovium is likely to occur through pattern-recognition receptors (PRRs), such as Toll-like receptor 2 (TLR2), TLR3, TLR4 and TLR6, which recognize various microbial products, as well as putative endogenous ligands, including heat-shock proteins and fibronectin[57,58]. TLR expression is established in chronic disease and as such could serve not only to initiate but also to perpetuate disease. TLR signalling is implicated in rodent models of arthritis; TLR2- and TLR4-deficient strains show reduced CIA and streptococcal-cell-wall-induced arthritis[59]. Similarly, cytokine expression in human synovial *in vitro* cultures is promoted through pathways that depend on the TLR signalling adaptor proteins MyD88 (myeloid differentiation primary-response gene 88) and TIRAP (Toll/IL-1-receptor (TIR)-domain-containing adaptor protein; also known as MAL)[60]. Synovial monocytes can also be activated to produce cytokines by immune complexes through their cell-surface FcγRs. Finally, the synovial membrane is rich in serine proteases. Recent studies indicate that the synthesis of proteases, such as mast-cell tryptase and trypsin, by neutrophils and mast cells, might regulate macrophage cytokine release by activating protease-activated receptor 2 (PAR2; also known as F2RL1). Consistent with this, PAR2-deficient mice exhibit reduced inflammation in adjuvant- and antigen-induced models of arthritis[61], and specific inhibition of PAR2 in rheumatoid synovial cultures directly inhibits endogenous cytokine synthesis[62].

The key cytokines that are released by synovial macrophages and their relevant effects are shown in TABLE 1. Their integrated biology is shown in FIG. 3. TNF is clearly of primary importance in the pathogenesis of rheumatoid arthritis[63,64]. TNF is present in most synovial biopsies, and its inhibition suppresses various arthritis models, whereas overexpression of a *TNF* transgene induces spontaneous erosive inflammatory arthritis[65]. TNF induces leukocyte and endothelial-cell activation, synovial-fibroblast activation and survival, pain-receptor sensitization and

Follicular DCs
These are specialized non-haematopoietic stromal cells that reside in the follicles and germinal centres. These cells possess long dendrites, but are not related to dendritic cells, and carry intact antigen on their surface.

Germinal centres
Highly specialized and dynamic microenvironments that give rise to secondary B-cell follicles during an immune response. They are the main site of B-cell maturation, leading to the generation of memory B cells and plasma cells that produce high-affinity antibody.

SCID mouse
Severe combined immunodeficiency mouse. Mice of this phenotype lack functional T and B cells owing to a spontaneous mutation in the *Prkdc* gene (protein kinase, DNA activated, catalytic polypeptide) located on chromosome 16. These mice are often used for reconstitution of T-cell subsets to study their functions *in vivo*.

Affinity maturation
A process whereby the mutation of antibody variable (V)-region genes followed by selection for higher-affinity variants in the germinal centre leads to an increase in average antibody affinity for an antigen as an immune response progresses. The selection is thought to be a competitive process in which B cells compete with free antibody to capture decreasing amounts of antigen.

Receptor editing
A molecular process that involves secondary rearrangements (mostly of the light chains) that replace existing immunoglobulin molecules and generate a new antigen receptor with altered specificity.

Mantle zone
The area of a secondary follicle that surrounds the germinal centre and contains IgD^+ naive, resting B cells.

Table 1 | **Selected key cytokine activities implicated in the pathogenesis of rheumatoid arthritis**

Cytokine	Articular cell expression	Potential functions in the pathogenesis of rheumatoid arthritis
IL-1α and/or IL-1β	Monocytes, B cells, synovial fibroblasts, chondrocytes	↑Synovial fibroblast cytokine, chemokine, MMP, iNOS and PG release; ↑monocyte cytokine, ROI and PG release; osteoclast activation; ↓GAG synthesis, ↑iNOS, MMP and aggrecanase; endothelial-cell adhesion molecule expression
IL-18	Monocytes, PMNs, DCs, platelets, endothelial cells	T-cell differentiation (T_H1 cells with IL-12; T_H2 cells with IL-4); NK-cell activation, cytokine release and cytotoxicity; ↓chondrocyte GAG synthesis, iNOS expression; monocyte cytokine release and adhesion molecule expression; PMN activation, cytokine release and migration; pro-angiogenic for endothelial cells
TNF	Monocytes, T cells, B cells, NK cells, PMNs, mast cells, synovial fibroblasts, osteoblasts	↑Monocyte activation, cytokine and PG release; ↑PMN priming, apoptosis and oxidative burst; T-cell apoptosis, clonal regulation and TCR dysfunction; ↑endothelial-cell adhesion molecule expression, cytokine release; ↓synovial fibroblast proliferation and collagen synthesis, ↑MMP and cytokine release; ↑adipocyte FFA release; endocrine effects
LTα and/or LTβ	T cells, monocytes, synovial fibroblasts	Peripheral lymphoid organ development; otherwise similar bioactivities to TNF
RANKL	Stromal cells, osteoblasts, T cells	Stimulates bone resorption via osteoclast maturation and activation; modulates T-cell–DC interactions
BAFF	Monocytes, T cells, DCs	B-cell proliferation, antibody secretion, isotype switching and survival; T-cell co-stimulation
APRIL	Monocytes, T cells	B-cell proliferation
IL-17A	T_H17 cells, synovial fibroblasts	↑Synovial fibroblast cytokine and MMP release; osteoclastogenesis; haematopoiesis; ↓chondrocyte GAG synthesis; ↑leukocyte cytokine production
IL-12	Macrophages, DCs	T_H1-cell proliferation and maturation; T-cell and NK-cell cytotoxicity; B-cell activation
IL-23	Macrophages, DCs	T_H17-cell proliferation
IL-7	Synovial fibroblasts, monocytes?	T-cell expansion and survival; macrophage activation; haematopoietic regulation; thymic regulation; NK-cell maturation
IL-15	Monocytes, synovial fibroblasts, mast cells, B cells, PMNs, DCs	T-cell chemokinesis, activation and memory maintenance; B-cell differentiation and isotype switching; NK-cell activation and cytotoxicity; synovial fibroblast activation; macrophage activation/suppression (dose dependent); PMN activation, adhesion molecule expression and oxidative burst
IL-10	Monocytes, T cells, B cells DCs, epithelial cells	↑Macrophage cytokine release, iNOS and soluble receptor expression, ↓ROI; T-cell cytokine release, ↓MHC expression, anergy induction, T_{Reg}-cell maturation and effector function(?); ↓DC activation and cytokine release; ↓synovial fibroblast MMP and collagen release; ↑B-cell isotype switching
IL-6	Monocytes, synovial fibroblasts, B cells, T cells	B-cell proliferation and antibody production; haematopoiesis and thrombopoiesis; T-cell proliferation, differentiation and cytotoxicity; ↑hepatic acute-phase response; ↑neuroendocrine effects
Oncostatin M	Monocytes, activated T cells	Megakaryocyte differentiation; ↑synovial fibroblast TIMP and cytokine release, ↑acute-phase reactants, ↑protease inhibitors; ↓monocyte TNF release, ↓IL-1 effector function; ↑neuroendocrine effects and corticosteroid release; osteoblast modulation(?)
TGFβ	Synovial fibroblasts, monocytes, T cells, platelets	Wound repair, matrix maintenance and fibrosis; T_H17- and T_{Reg}-cell proliferation; ↓NK-cell proliferation and effector function; initial activation then suppression of inflammatory responses; ↑early phase leukocyte chemoattractant, gelatinase and integrin expression; early macrophage activation then suppression; ↓ iNOS expression
BMP family (BMP2–BMP15)	Epithelial cells, synovial fibroblasts, mesenchymal embryonic tissues	Regulate crucial chemotaxis, mitosis and differentiation processes during chondrogenesis and osteogenesis; tissue morphogenesis
PDGF	Platelets, macrophages, endothelial cells, synovial fibroblasts	Paracrine and/or autocrine growth factor for various lineages; wound healing
FGF family	Synovial fibroblasts, monocytes	Growth and differentiation of mesenchymal, epithelial and neuroectodermal cells
VEGF	Monocytes, endothelial cells, synovial fibroblasts	Angiogenesis
IL-32	Epithelial cells, monocytes(?), synovial fibroblasts(?)	Macrophage cytokine, PG and MMP release
MIF	Macrophages, activated T cells, synovial fibroblasts	↑Macrophage phagocytosis, cytokine and NO release; T-cell activation, DTH; fibroblast proliferation, COX expression, PLA_2 expression and intrinsic oxidoreductase activity ('cytozyme')
Type I IFNs	Widespread	Antiviral response; broad immunomodulatory effects; ↑MHC expression; macrophage activation; lymphocyte activation, differentiation, survival (antiproliferative) and cytoskeletal alterations

This table is adapted and updated from several tables contained in REF. 129. APRIL, a proliferation-inducing ligand; BAFF, B-cell activating factor; BMP, bone morphogenetic protein; COX, cyclooxygenase; DTH, delayed-type hypersensitivity; DC, dendritic cell; FFA, free fatty acid; FGF, fibroblast growth factor; GAG, glycosaminoglycans; IFN, interferon; IL, interleukin; iNOS, inducible nitric-oxide synthase; LT, lymphotoxin; MIF, macrophage migration-inhibitory factor; MMP, matrix metalloproteinase; NK, natural killer; PDGF, platelet-derived growth factor; PG, prostaglandin; PLA_2, phospholipase A_2; PMN, polymorphonuclear leukocyte; RANKL, receptor activator of nuclear factor-κB (RANK) ligand; ROI, reactive oxygen intermediate; TCR, T-cell receptor; TGFβ, transforming growth factor-β; T_H, T helper; TIMP, tissue inhibitor of MMPs; TNF, tumour-necrosis factor; T_{Reg}, regulatory T; VEGF, vascular endothelial growth factor.

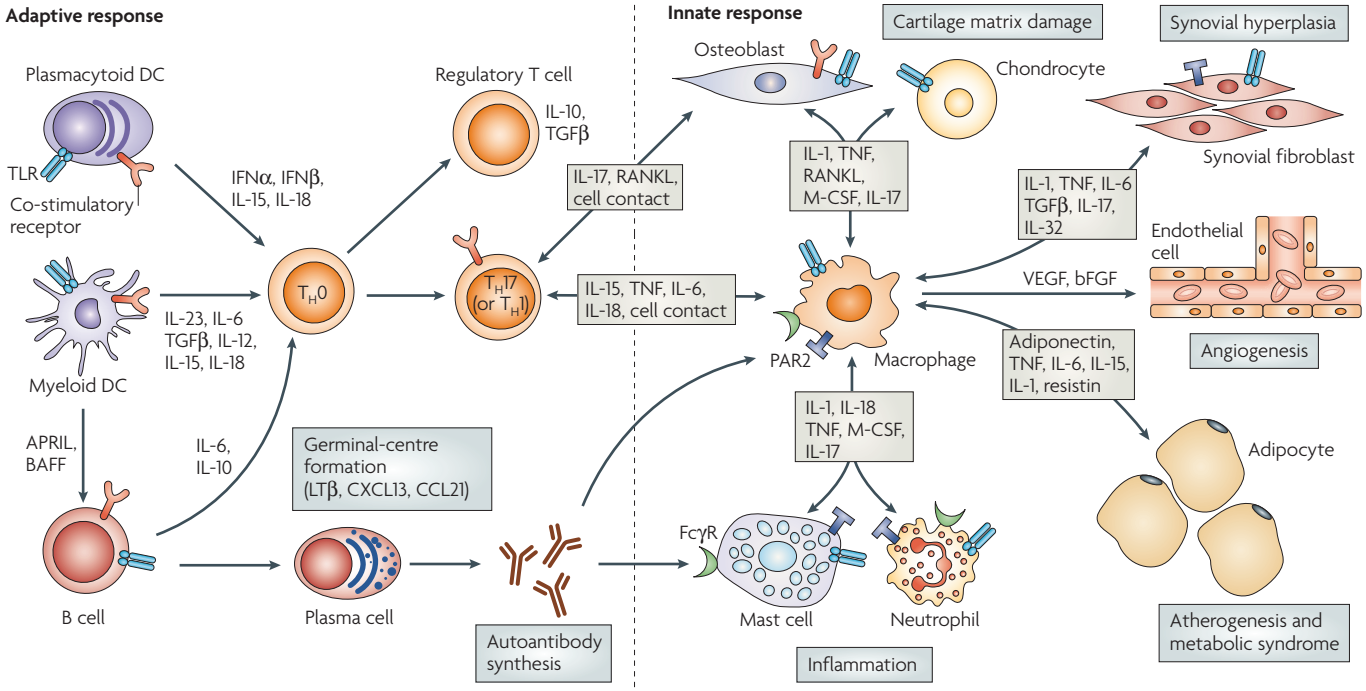

Figure 3 | **An overview of the cytokine-mediated regulation of synovial interactions.** The component cells of the inflamed rheumatoid synovial membrane are depicted in innate and adaptive predominant compartments of the inflammatory response. Pivotal cytokine pathways are depicted in which activation of dendritic cells (DCs), T cells, B cells and macrophages underpins the dysregulated expression of cytokines that in turn drive activation of effector cells, including neutrophils, mast cells, endothelial cells and synovial fibroblasts. The clinical manifestations of such effects are highlighted. Only key cytokines are shown in each domain for relative simplicity; the main text contains more detailed description of the precise role of additional cytokines in these processes. Bidirectional arrows represent a relationship between cells that is influenced by the cytokines listed. The pathways that lead to tissue destruction via osteoclast and chondrocyte activation are detailed in FIGS 4,5. APRIL, a proliferation-inducing ligand; BAFF, B-cell activating factor; bFGF, basic fibroblast growth factor; CCL21, CC-chemokine ligand 21; CXCL13, CXC-chemokine ligand 13; FcγR, Fc receptor for IgG; IFN, interferon; IL, interleukin; LTβ, lymphotoxin-β; M-CSF, macrophage colony-stimulating factor; PAR2, protease-activated receptor 2; RANKL, receptor activator of nuclear factor-κB (RANK) ligand; TGFβ, transforming growth factor-β; T_H, T helper; TLR, Toll-like receptor; TNF, tumour-necrosis factor; VEGF, vascular endothelial growth factor.

angiogenesis, which together represent key pathological features of rheumatoid arthritis. Therapeutic blockade of TNF yields clinical responses in approximately 70% of patients with established rheumatoid arthritis, and it results in a rapid (<24 hrs) decrease in plasma levels of IL-6 and acute-phase proteins, suppression of leukocyte migration, endothelial-cell deactivation[2] and recovery of regulatory T-cell function and phenotype[31]. Notably, intervention with TNF inhibitors early in the disease is most effective[66], which raises the possibility that immune dysregulation is reversible, but perhaps only when joint damage is minimal and there are therefore fewer endogenous danger signals.

Other cytokines are emerging at critical points in the regulatory hierarchy. IL-6 is an important monocyte-derived effector cytokine. It manifests plausible effector function in synovial membranes *in vitro* (TABLE 1) and is required for the induction of CIA. Similarly, IL-15 exhibits pro-inflammatory activity *in vitro*[12] and in animal models of arthritis[67], and may represent a good therapeutic target[68]. IL-1α and IL-1β are expressed in the synovium of patients with rheumatoid arthritis[69], and mice deficient in IL-1 receptor antagonist (IL-1RA; also known as IL1RN) develop spontaneous erosive arthritis associated with the induction of T_H17 cells[69,70]. The administration of recombinant IL-1RA is effective in disorders that are driven by IL-1 dysregulation, such as the cold autoinflammatory diseases[71]. However, therapeutic targeting of the IL-1 pathway has elicited clinical responses of modest magnitude in rheumatoid arthritis. Use of the adaptor protein MyD88 downstream of both IL-1R and TLR4 provides the possibility that endogenous synovial TLR ligands could bypass IL-1-dependent signalling and offer an explanation for these clinical findings in rheumatoid arthritis; this is not yet proven in human tissues[72]. Interest in the IL-1-family members has also focused on IL-18, which is expressed and functionally active in rheumatoid synovium[73,74]. New members of this superfamily, such as IL-F5–IL-F10 and IL-33, remain under investigation. Finally, IL-32 is an IL-18 inducible, inflammatory cytokine that has been recently identified in synovial macrophages. IL-32γ promotes prostaglandin-E_2 synthesis *in vitro* and after its injection *in vivo* it induces TNF-dependent joint inflammation but TNF-independent matrix degradation[75]. IL-32γ synergizes with ligation of the intracellular PRR NOD2

Danger signals
Agents that alert the immune system to danger, usually by interacting with Toll-like receptors and other pattern-recognition receptors, and thereby promote the generation of innate and adaptive immune responses. Danger signals can be associated with microbial invaders (exogenous danger signals) or produced by damaged cells (endogenous danger signals).

IL-1 receptor antagonist
(IL-1RA). A secreted protein that binds to the interleukin-1 receptor (IL-1R), thereby blocking IL-1R downstream signalling. IL-1RA inhibits the pro-inflammatory properties of IL-1α and IL-1β.

(nucleotide-binding oligomerization domain protein 2) to promote cytokine release by macrophages[76]. Notably synovial expression of IL-32 correlates with synovial levels of TNF and IL-1β, and with the erythrocyte sedimentation rate, a marker of systemic inflammation[75].

Two other cytokines merit mention by virtue of their distinctive biological features. High-mobility group box 1 protein (HMGB1), which was originally defined as a nuclear transcriptional factor, has been shown to be released from cells and have pro-inflammatory activities[77]. Blockade of HMGB1 using specific antibodies or the A-box protein suppresses CIA[77]. It is implicated directly in cartilage degradation, rendering it a potential link between inflammation and joint damage. Macrophage migration-inhibitory factor (MIF) is a potent pro-inflammatory cytokine that drives synovial macrophage release of cytokines and prostaglandins and is a regulator of synovial fibroblast survival in a p53-dependent manner[78]. There is particular interest in the cytokine MIF because it has an intriguing autocrine role in glucocorticoid function, such that its inhibition could serve as a means to reduce steroid use in patients with rheumatoid arthritis[78].

Other innate immune effector cells and cytokines. Recent data increasingly implicate other innate immune response effector pathways, particularly those driven by type I IFNs; IFNα and IFNβ are expressed in rheumatoid synovitis[64]. Moreover, genomic profiling studies further suggest that type I IFN responses may define clinically distinct subsets of patients with rheumatoid arthritis[79] — the prognostic and therapeutic implications of such observations require further definition but support the notion that innate immune pathways remain of critical importance throughout the course of rheumatoid disease.

Neutrophils are present in high numbers in synovial fluid and traffic through the synovial membrane. Although their primary role in the pathology of this disease is disputed, they synthesize a wide variety of cytokines, including TNF, IL-1, IL-18, IL-15, IL-6 and BAFF, and therefore could support a range of pathological events. Neutrophils are activated by immune complexes, complement components and cytokines to release chemokines, prostaglandins, reactive oxygen intermediates and reactive nitrogen intermediates, and therefore also probably contribute significantly to the general hypoxic milieu in inflamed joints (reviewed in REF. 80).

Mast cells are similarly implicated at the crossroad of innate and adaptive synovial immunity. They are widely distributed in rheumatoid arthritis synovial tissue and express various proteases and pro-inflammatory cytokines (reviewed in REF. 81). Studies involving the transfer of arthritogenic serum from the K/BxN-transgenic mouse model to mast-cell-deficient (*W/Wv*) mice clearly demonstrate integration of immune-complex-, complement- and cytokine- (particularly IL-1 and TNF) mediated synovial inflammation[82]. Mast-cell targeting is enticing in the clinic. Intriguing recent pre-clinical studies demonstrate the beneficial effects of targeting mast-cell signalling in CIA by treatment with imatinib mesylate (Gleevec; Novartis), a protein tyrosine kinase inhibitor. Such effects were mediated, in part, by inhibition of signalling by the receptor tyrosine kinase KIT in mast cells, and also by inhibition of signalling by macrophage colony-stimulating factor receptor (M-CSFR; also known as FMS and CSF1R) in macrophages and by platelet-derived growth-factor receptor in synovial fibroblasts[83].

NK-cell subsets are widely distributed within the rheumatoid synovial membrane and could constitute a significant cytokine source[84]. Although NK-cell activation by cytokines, including IL-15, IL-18 and IL-12, leads to increased NK-cell cytotoxic activity and release of cytokines, such as TNF and IFNγ, the role of NK cells in synovial inflammation remains poorly understood.

Inhibitory cytokines and receptors in synovitis

One important feature of rheumatoid synovitis is the relative expression deficiency of several regulatory cytokines, thereby contributing to the imbalance between pro-inflammatory and anti-inflammatory cytokines in the joints. Therefore, although IL-10, IL-11 and IL-1RA are expressed by synovial mononuclear cells, they are not present in sufficient local concentrations to mediate counter-regulatory activity against the dominant pro-inflammatory cytokine milieu[64]. The T-cell-derived cytokines IL-2 and IL-4 are similarly absent[64], which may impair T_{Reg}-cell generation and favour T_H1-cell or T_H17-cell differentiation, respectively. Soluble cytokine receptors, such as TNFR1 and the type II IL-1R, which have a regulatory role in sequestering soluble TNF and IL-1 away from their cell-bound receptors, can be readily detected in synovial tissue and fluid but are insufficient to mediate tissue homeostasis[64]. Direct replenishment of such activities by systemic therapeutic administration of IL-10 or IL-1RA, for example, has offered only modest success in practice so far[85]. However, local gene-delivery vehicles may offer more beneficial opportunities in the future.

Cytokine-mediated articular destruction

Osteoclasts and inflammatory bone erosion. Inflammation and bone erosion are closely linked[86–88]. Normal physiological processes ensure a balance between bone formation and bone resorption to maintain skeletal homeostasis. This balance is perturbed in rheumatoid arthritis in favour of bone resorption. Bone resorption depends on osteoclasts[89]. Mice with a differentiation defect in the osteoclast lineage (such as mice lacking FOS) develop inflammatory arthritis that is not associated with bone erosion[90]. In rheumatoid arthritis, osteoclasts at the interface between synovial tissue and articular bone induce bone resorption, which in turn permits invasion by cells of the synovial membrane and results in pannus formation[91]. This process depends on the influx of osteoclast precursors into inflamed synovial tissue and the differentiation of these cells into mature osteoclasts. Their metabolic activation to resorb bone requires complex cellular interactions between cells of the osteoclast lineage with mesenchymal cells and lymphocytes. These interactions are controlled by cytokines (FIG. 4).

M-CSF and RANKL (receptor activator of nuclear factor-κB (RANK) ligand; also known as TNFSF11) are essential for the differentiation of osteoclasts from

K/BxN transgenic mouse
A mouse strain formed by crossing NOD/Lt mice with C57BL/6 × KRN T-cell-receptor-transgenic mice in which T cells recognize a peptide from the autoantigen glucose-6-phosphate isomerase (GPI). These mice develop an arthritis that is mediated, and transferable, by circulating antibody against GPI.

Pannus
A sheet of inflammatory granulation tissue, composed of immune cells, blood vessels and fibrous cells, that spreads from the synovial membrane and ultimately invades the joint in rheumatoid arthritis.

their precursor cells, and a lack of either molecule is sufficient to block osteoclast formation completely[92]. M-CSF is expressed by synovial mesenchymal cells and to a lesser extent by T cells[93]. TNF induces the production of M-CSF by synovial fluid cells, as does IL-7, which promotes M-CSF production by T_H1 cells. M-CSF engages its receptor M-CSFR on monocytes, inducing early differentiation into osteoclasts. The M-CSF–M-CSFR interaction is essential for osteoclastogenesis but alone is insufficient to induce their final differentiation.

RANKL, a member of the TNF superfamily, is expressed by mesenchymal cells, such as synovial fibroblasts, and activated synovial T cells[94]. In arthritis models and rheumatoid synovial tissue, RANKL expression is upregulated and constitutes an important prerequisite for osteoclast differentiation[91,95,96]. RANKL expression is regulated by inflammatory cytokines, such as TNF, IL-1β, IL-6 and IL-17, but is also influenced by non-cytokine inflammatory mediators such as prostaglandin E_2 (REFS 97,98). RANKL induces the final differentiation of osteoclasts and their bone-resorbing activity. The interaction of RANKL with its receptor RANK is modulated by osteoprotegerin (OPG; also known as TNFRSF11B), a soluble decoy receptor, which is expressed by mesenchymal cells in the rheumatoid arthritis synovium[99]. In rheumatoid arthritis, an imbalance between OPG and RANKL expression promotes RANKL-induced bone loss. In mice, exogenous OPG expression completely blocks arthritic bone erosions[95].

Other cytokines in the inflammatory milieu also contribute to the destructive process. TNF is a potent driver of osteoclast formation, acting either additively with RANKL[100] or directly through TNFRI. TNF also mobilizes $CD11b^+$ osteoclast precursors from the bone marrow[101,102]. IL-1β induces RANKL expression and acts additively with RANKL in driving osteoclastogenesis. Moreover, IL-1β is a key component of TNF-mediated osteoclastogenesis; TNF induces IL-1 and IL-1R expression by mesenchymal cells and cells of the osteoclast lineage, which subsequently support the RANKL–RANK system[103]. IL-1β also regulates RANK expression (J. Zwerina, unpublished observations). IL-17 induces RANKL, TNF and IL-1β expression by synovial fibroblasts to support osteoclast formation. T_H17-cell-inducing cytokines, including IL-23, TGFβ and IL-6, therefore, probably act on osteoclasts via this pathway[104]. Although T_H17 and T_H1 cells express RANKL, the relative impact of this pathway in inflammatory bone loss *in vivo* is unclear[105]. RANKL expression by T_H1 cells is considered a key link in osteoimmunology, providing an explanation to why immune activation is linked to bone loss. However, stimulation of T_H1 cells by IL-18 or IL-12 induces expression of IFNγ and GM-CSF, which both suppress osteoclast differentiation[106,107].

Bone formation, which is the 'physiological' counter-regulatory response to increased bone resorption is mediated by osteoblasts and is virtually absent in rheumatoid arthritis. Although the regulation of bone formation in rheumatoid arthritis is poorly defined, cytokines are likely to be important. For example, TNF inhibits osteoblast differentiation and function[108]. TNF upregulates secretory molecules, such as dickkopf homologue 1 (DKK1), which acts as a WNT-protein inhibitor and blocks bone and cartilage formation[109].

Osteoblasts
Cells of mesenchymal origin that are responsible for the formation of bone.

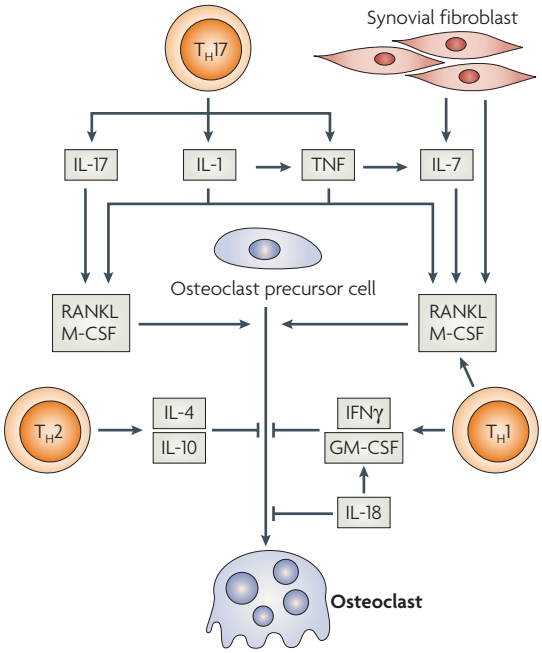

Figure 4 | **The key factors that regulate osteoclast differentiation in rheumatoid arthritis.** The differentiation of bone-resorbing osteoclasts from haematopoietic osteoclast precursors is a cytokine-driven process. The essential cytokine mediators are RANKL (receptor activator of nuclear factor-κB (RANK) ligand) and M-CSF (macrophage colony-stimulating factor), which are expressed by synovial fibroblasts and T helper 1 (T_H1) cells. Osteoclast differentiation is achieved by the actions of tumour-necrosis factor (TNF) and interleukin-1 (IL-1), as well as of IL-17, produced by T_H17 cells, and IL-7, produced by synovial fibroblasts. By contrast, IL-4 and IL-10, which are produced by T_H2 cells, and granulocyte/M-CSF (GM-CSF) and interferon-γ (IFNγ), which are produced by T_H1 cells, inhibit osteoclast differentiation.

Synovial fibroblasts integrate inflammatory signals in articular damage. In rheumatoid joints, synovial fibroblasts exhibit anchorage-independent growth, loss of contact inhibition and increased proliferation, and play a central role in chronic synovitis[110]. They form a cadherin-11-dependent lining layer in the synovial membrane, the presence of which is required for optimal inflammation-driven erosive disease *in vivo*. Cadherin-11-deficient mice display a hypoplastic synovial lining layer, which prevents mesenchymal-cell responses, such as synovial hyperplasia during inflammation[111]. The mechanism of synovial hyperplasia is incompletely understood but exposure to cytokines, such as basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF) and TGFβ, together with the induction of expression of oncogenes, such as *RAS* and *MYC*, and survival proteins, such as heat-shock protein 70 (HSP70), sentrin and sumoylated proteins, appear to be crucial[112]. Activation of

synovial fibroblasts is also initiated by cytokines, among which TNF and IL-1β are paramount. Synovial fibroblasts in turn produce TNF, IL-1β and IL-6 to sustain regulatory feedback loops that induce the production of matrix enzymes, such as MMPs, aggrecanases and cathepsins, as well as their inhibitors. This enzymatic milieu contributes to the local migratory activity and crucially serves as a prerequisite for articular cartilage invasion. Synovial fibroblasts promote T-cell and B-cell migration, activation and survival by expressing the requisite chemokines and cytokines (such as TNF, IL-7, IL-15, IL-16 and BAFF)[113]. In turn, B cells promote synovial-fibroblast activation through IgG binding to the high-affinity FcγR (FcγRI) on synovial fibroblasts[114]. The production of vascular endothelial growth factor (VEGF), bFGF, oncostatin M and IL-18 by synovial fibroblasts promotes angiogenesis. Together these data indicate a central role for synovial fibroblasts in integrating the inflammatory and destructive phases of inflammatory arthritis critically regulated by cytokines.

Cartilage degradation. Articular cartilage is composed of a non-mineralized surface layer and a deep mineralized layer adjacent to bone. Only the resorption of the mineralized layer is osteoclast mediated. Both layers contain chondrocytes, which determine cartilage metabolism. Two mechanisms lead to the disintegration of undifferentiated cartilage (FIG. 5). First, chondrocytes switch from an anabolic matrix-synthesizing state to a catabolic state that is characterized by the formation of ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) and MMPs that cleave the cartilage components proteoglycan and collagen fibres, respectively. The chondrocytes themselves synthesize cytokines or respond to local cytokine release, particularly IL-1β, IL-17, IL-18 and TNF, to accelerate the switch from an anabolic to a catabolic state. Second, matrix-degrading enzymes are also released by synovial fibroblasts, neutrophils and potentially mast cells, which are closely located to articular cartilage.

There seems to be a hierarchy of cytokine effects on cartilage. IL-1β plays a central role in cartilage degradation through the inhibition of matrix synthesis, as well as through the induction of expression of the matrix-degrading enzymes MMP1, MMP3, MMP8, MMP13 and MMP14 (REFS 115,116). IL-1-driven animal models of arthritis, such as CIA and IL-1RA-deficient mice, show rapid and extensive cartilage damage[117]. Injection of IL-1 intra-articularly leads to proteoglycan loss, chondrocyte apoptosis and matrix degradation[118]. By contrast, TNF seems to be a less potent inducer of matrix-enzyme expression than IL-1. Even in TNF-driven disease, cartilage damage may be mediated exclusively by downstream IL-1 (J. Zwerina and G.S., unpublished observations). IL-18 also operates via IL-1β to degrade cartilage[119]. IL-17 acts directly and synergistically with IL-1β and TNF to promote cartilage catabolism. Intra-articular injection of IL-17 increases proteoglycan loss but does not progress to erosion and chondrocyte death[120]. However, following induction of CIA, exogenous IL-17 strongly enhances cartilage damage and leads to erosions and chondrocyte death[121]. These effects appear to require immune complexes, which facilitate the binding of cells to cartilage and the production of ADAMTS5 and MMP3 in close proximity to the matrix. IL-17 can lead to cartilage degradation in IL-1β-deficient mice, which indicates that IL-17 can bypass IL-1 for bone destruction. Moreover, as TNF uses IL-1β to degrade cartilage, it is conceivable that IL-17 does not require TNF for cartilage damage *per se* but acts together with TNF. The precise mechanism by which IL-17 directly precipitates cartilage damage is not fully clarified, but probably involves the leukaemia inhibitory factor (LIF)-dependent induction of prostaglandin E_2 and nitric oxide in chondrocytes[122].

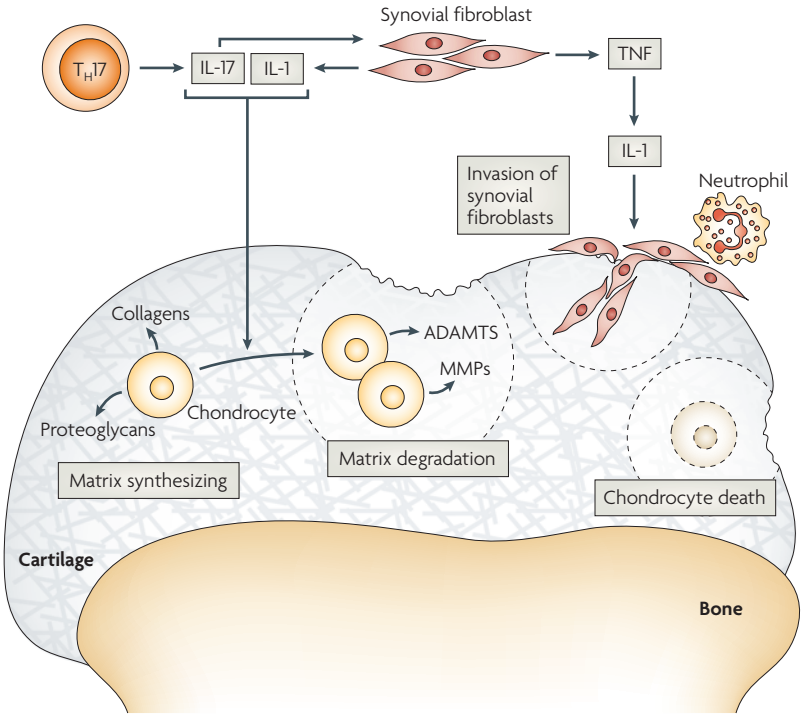

Figure 5 | **Pathways regulating chondrocyte activation and cartilage degradation in rheumatoid arthritis.** Cartilage degradation is a multistep process based on the release of matrix-degrading enzymes such as aggrecanases (ADAMTS) and matrix metalloproteinases (MMPs). Cytokines such as interleukin-1 (IL-1) and IL-17 induce a switch in the synthesis pattern of chondrocytes from matrix molecules to matrix-degrading enzymes. In addition, synovial fibroblasts start producing matrix-degrading enzymes and invade cartilage when activated by cytokines such as tumour-necrosis factor (TNF) and IL-1. Chondrocyte death is another feature of cartilage damage, it leads to the formation of empty lacunae and deprives cartilage from the ability to replenish matrix.

Angiogenesis as a crucial requirement for articular destruction? Synovial cytokines are implicated in the profuse angiogenetic activity that is typical of the rheumatoid synovium, and this in turn is a prerequisite for inflammation and destruction. Therefore, the pro-angiogenic factors VEGF and bFGF, and their respective receptor complexes are expressed and functional in the synovial membrane (reviewed in REF. 123). Inhibition of angiogenesis suppresses synovitis *in vitro* and *in vivo*.

Diapedesis
The migration of leukocytes across the endothelium, which occurs by leukocytes squeezing through the junctions between adjacent endothelial cells.

Additional cytokines are implicated, particularly TNF, as clinical inhibition of TNF is associated with the suppression of neovascularization[124]. The clinical role of cytokine inhibitors of angiogenesis remains unclear but offers theoretical promise.

Chemokines in synovitis

For many years, rheumatoid synovitis has been known to involve a large number of chemokines[125]. Accordingly, synovial immune and mesenchymal cells express discrete patterns of reciprocal chemokine receptors. As described above, regulatory chemokines, such as CCL2, CCL5, CCL21 and CXCL13, contribute to synovial lymphoid organization. In addition, several other important roles have been ascribed to chemokines in synovitis, including leukocyte recruitment, diapedesis, activation and retention within the inflamed tissue. In all of these activities, they function together with cytokines that regulate their expression and that of their reciprocal receptors. Chemokine expression and their functional importance in rheumatoid arthritis have provoked several therapeutic targeting studies, although with limited success so far. For further information on the role of the chemokine pathway in rheumatoid arthritis, see the recent elegant reviews[126,127].

Challenges and opportunities

TNF blockade has proved successful in the treatment of rheumatoid arthritis and many other autoimmune inflammatory disorders; however, a significant proportion of patients show only partial responses or fail to respond. Current clinical data suggest that inhibition of IL-6 and IL-15 (REFS 7,68), and perhaps IL-12, IL-23, IL-18 and IL-17 (REF. 74), could offer therapeutic potential (TABLE 2). Choosing the correct target is not easy. The hierarchical relationships within the synovial cytokine network remain unclear. A linear model was originally proposed in which TNF would drive downstream cytokines, such as IL-1 and IL-6, sequentially, and this model seemed to explain the dominance of TNF in synovial culture systems[63]. However, the detection of complex cytokine expression patterns and variable expression of TNF in individual joints raises the possibility of parallel cytokine pathways with ongoing crosstalk at multiple levels, which facilitate the bypass of single pathways upon therapeutic neutralization. This predicts partial or lack of responses across a range of therapeutic targets that define distinct rheumatoid arthritis subgroups. Future studies must identify which cytokines, beyond TNF, operate as 'checkpoints' in synovial inflammation; clinical studies suggest that IL-6 and perhaps IL-15 could serve this role in some patients. Relating synovial cytokine expression patterns to the natural progression of the disease will be essential.

Testing hypotheses reliably in pre-clinical studies remains challenging. The pre-clinical drug development model, using tissue cytokine expression, *in vivo* models and proof-of-concept clinical studies, is pragmatic but arguably not robust, nor efficient. Novel model systems, including *in silico* approaches, are urgently required to rationalize target selection and prioritization. Commensurate with this, mode-of-action studies should be mandatory in Phase I clinical trials, and this would provide opportunities to

Table 2 | **Selected cytokines as therapeutic targets in rheumatoid arthritis**

Cytokine	Advantages as a target	Disadvantages as a target	Development stage	Agent(s)
TNF	Plausible bioactivity *in vitro* and in models; validated clinical target; efficacy in approx 70% of recipients	Infection risk (such as tuberculosis); possible increased malignancy	Widespread clinical use	Infliximab*, adalimumab‡ (TNF-specific antibodies); etanercept§ (TNF receptor–Fc fusion protein)
IL-1	Plausible bioactivity *in vitro* and in models; particular role in matrix degradation	Limited efficacy in clinical trials; infection risk	Licensed for clinical use	Anakinra¶ (recombinant IL-1RA)
IL-6	Plausible bioactivity *in vitro* and in models; good efficacy so far in clinical trials	Essential role in host defence? Lipid and vascular modification?	Phase III clinical trials	Tocilizumab# (IL-6-receptor-specific antibody)
IL-12 or IL-23	Plausible bioactivity in models; role in T_H1- and/or T_H17-cell expansion; role in breach of tolerance?	Limited investigation in synovial biology; essential role in host defence?	Pre-clinical or proof of concept	Antibodies specific for p40, antibodies specific for p19
IL-15	Plausible bioactivity *in vitro* and in models; trends to efficacy in early clinical trials; role in breach of tolerance?	Essential role in host antiviral responses? Essential role in NK-cell biology?	Phase II clinical trials	AMG714**
GM-CSF	Plausible bioactivity *in vitro* and in models	Unclear hierarchical priority in rheumatoid arthritis	Pre-clinical or proof of concept	Antibody specific for cytokine or receptor
IL-17	Plausible bioactivity *in vitro* (synergy with TNF); key role in rodent models of autoimmunity	Human biology requires clarification; essential role in host defence?	Phase I clinical trials	Antibody specific for cytokine
IL-18	Plausible bioactivity *in vitro*	Ambiguous *in vivo* targeting; essential role in host defence?	Phase I clinical trials	Antibody specific for cytokine, IL-18-binding protein

*Remicade; Centocor. ‡Humira; Abbott Laboratories. §Enbrel; Amgen, Wyeth. ¶Kineret; Amgen. #Actemra; Chugai, Roche. **Amgen. GM-CSF, granulocyte/macrophage colony-stimulating factor; IL, interleukin; IL-1RA, IL-1 receptor antagonist; NK, natural killer; T_H, T helper; TNF, tumour-necrosis factor.

study disease pathogenesis. Moreover, there is a need to identify predictive biomarkers for the appropriate selection of targets and patients a priori, and for predicting therapeutic responses.

The induction of disease remission is now the therapeutic goal. Targeting cytokines that regulate autoimmune T-cell activation and cell-fate determination during the very early stages of the disease may ultimately offer a means to induce tolerance. General suppression of inflammation at early stages of disease may offer a similar potential, such as through TNF blockade[128]. Finally, understanding cytokine signalling cascades may offer opportunities to target cytokine pathways with small-molecule inhibitors to replace the current trend to biological intervention

Here, we describe the cytokine network in terms of pathological processes. It will be crucial to select cytokine targets based not on one single inflammatory pathway but rather on a biosystematic approach to pathogenesis. Implicit in this will be the recognition of pivotal checkpoints that facilitate the progression from autoimmunity to chronic inflammation.

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