Review Article

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Temporal cytokine expression and the target organ attributes unravel novel aspects of autoimmune arthritis

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Susceptibility to autoimmunity is determined by multiple factors. Defining the contribution of the quantitative versus qualitative aspects of antigen-directed immune responses as well as the factors influencing target organ susceptibility is vital to advancing the understanding of the pathogenesis of autoimmunity. In a series of studies, we have addressed these issues using the adjuvant-induced arthritis (AA) model of human rheumatoid arthritis (RA). Lewis rats are susceptible to AA following immunization with heat-killed *Mycobacterium tuberculosis* H37Ra, whereas Wistar-Kyoto (WKY) rats of the same MHC (major histocompatibility complex) haplotype are resistant. Comparative studies on these and other susceptible/resistant rodent strains have offered interesting insights into differential cytokine responses in the face of comparable T cell proliferative response to the disease relevant antigens. Study of the cytokine kinetics have also permitted validation of the disease-protective versus disease-aggravating effects of specific cytokines by treatment of rats/mice with those cytokines at different phases of the disease. In regard to the target organ attributes, the migration of arthritogenic leukocytes into the joints; the expression of mediators of inflammation, angiogenesis, and tissue damage; the role of vascular permeability; and the characteristics of vascular endothelial cells have been examined. Further, various inhibitors of angiogenesis are effective in suppressing arthritis. Taken together, the differential cytokine responses and unique attributes of the target organ have revealed novel aspects of disease susceptibility and joint damage in AA. The translation of this basic research in animal models to RA patients would not only advance our understanding of the disease process, but also offer novel avenues for immunomodulation of this disease.

Key words Adjuvant arthritis - angiogenesis - arthritis - autoimmunity - cytokines - inflammation - joints - matrix metalloproteinases - regulatory T cells - T helper cells

Introduction

The immune system is capable of effectively responding to and containing a wide variety of pathogens (foreign; non-self), while guarding against immune response to the host tissues (self)[1-3]. However, certain constellations of genetic and environmental factors may result in a breakdown of self tolerance resulting in anti-self immune response (autoreactivity), which if not regulated, can result in immune pathology and dysfunction (autoimmunity). At the cellular level, the induction of autoimmunity is a manifestation of an imbalance between pathogenic effector versus protective regulatory responses. The manifestations of autoimmunity may either be systemic or organ-specific.

The traditional view of this dichotomy is based on the distribution of the antigen targeted by the autoimmune response, with widely-distributed autoantigens invoked in systemic diseases, while tissue-restricted self antigens implicated in organ-specific diseases. Although this scheme can explain the distribution of immune pathology in the body in many diseases, but it fails in other situations. The latter instances are those where the autoimmune response is directed against ubiquitously distributed antigens, yet the primary target of autoimmune damage may be limited to a particular tissue/organ[4,5].

It is increasingly being realized that the target antigen in organ-specific immunity may not necessarily be unique to that particular tissue/organ. Instead it may be distributed widely and yet the immune pathology may be predominantly focused on one or a few organs. This is illustrated in a couple of animal models of autoimmune arthritis. Adjuvant-induced arthritis (AA) in the rat is a well-studied model of human rheumatoid arthritis (RA)[6,7]. It can be induced in the Lewis rat by immunization with heat-killed *Mycobacterium tuberculosis* H37Ra (Mtb). AA is a T cell-mediated disease. Interestingly, immune response against mycobacterial heat-shock protein 65 (Bhsp65) has been implicated in the immunopathogenesis of AA[5,8-14]. Given the highly conserved nature of heat-shock proteins (Hsps), the T cells and antibodies directed against Bhsp65 are crossreactive with self hsp65 or other self ligands that mimic the foreign hsp65 epitopes. Further, Mtb also contains other heat-shock proteins besides Bhsp65. Hsp65 and other members of the Hsp60 family have been invoked not only in arthritis but also in multiple sclerosis (MS) and type I diabetes mellitus (T1D)[8,15-17]. However, Mtb-immunized Lewis rats develop arthritis without any concurrent autoimmune damage to the central nervous system or the pancreatic β-islet cells. The latter two represent the target organs in MS and T1D, respectively and their corresponding animal models are experimental autoimmune encephalomyelitis and the non-obese diabetic mice.

Another example of the animal model of arthritis in which the autoimmune response is directed against a ubiquitously distributed antigen is the K/BxN model of arthritis[4,18]. In this model, mice bearing a transgenic T cell receptor (TCR) specific for an epitope within ribonuclease, when crossed with non-obese diabetic (NOD) mice, develop spontaneous arthritis[18]. Interestingly, the above-mentioned TCR fortuitously crossreacts with a glycolytic enzyme, glucose 6-phosphate isomerase (GPI). Thus, spontaneous arthritis in these mice is the result of an autoimmune response against GPI, a widely distributed antigen.

The above examples relating to arthritis and similar ones involving other autoimmune diseases have given credence to the idea that the target organ attributes might play a vital role in their susceptibility to autoimmunity over and above the basic preconditions for the breakdown of self tolerance and the induction of autoreactivity. Broadly, the factors influencing the target organ susceptibility can be grouped into those that are extrinsic to that organ and others that are intrinsic. Extrinsic factors include, for example, the quantitative and qualitative aspects of the immune response generated in the peripheral lymphoid tissue draining the site of antigenic challenge or antigen encounter[12,19-21], and the kinetics of proinflammatory versus anti-inflammatory cytokines during the course of autoimmune arthritis[22,23]. Intrinsic factors include the angiogenic process associated with arthritis[24,25], the local vasculature and its permeability[4], the characteristics of the vascular endothelium of the joints[26], and the local release of immunological and biochemical mediators of tissue damage[27-30]. This article addresses specific examples of both extrinsic and intrinsic factors involved in the target organ damage in autoimmune arthritis. Most of the description is based on the rat AA model. However, at several places, examples from other animal models of arthritis have also been discussed. Further, some basic information has also been included on the subsets of T helper and regulatory T cells, the key pro-inflammatory cytokines, the inducers and regulators of angiogenesis, and the matrix metalloproteinases. All these cellular/soluble mediators play critical roles in the disease process in arthritis.

Subsets of T helper cells and regulatory T cells involved in the pathogenesis of autoimmunity

T helper cells: The majority of animal models of RA are T cell-mediated diseases[29] similar to the human disease[31]. The CD4+ helper T cells play an important role in the initiation and progression of acute inflammation in situations involving immune response to self (autoimmunity) or foreign (*e.g.* infectious disease) antigens. The cytokines produced by these cells help to facilitate the activation and chemotactic migration of other cell types to the site of inflammation during the immune response. The most widely studied helper CD4+ T cell types are the T helper 1 (Th1), Th2, and Th17. The cytokines

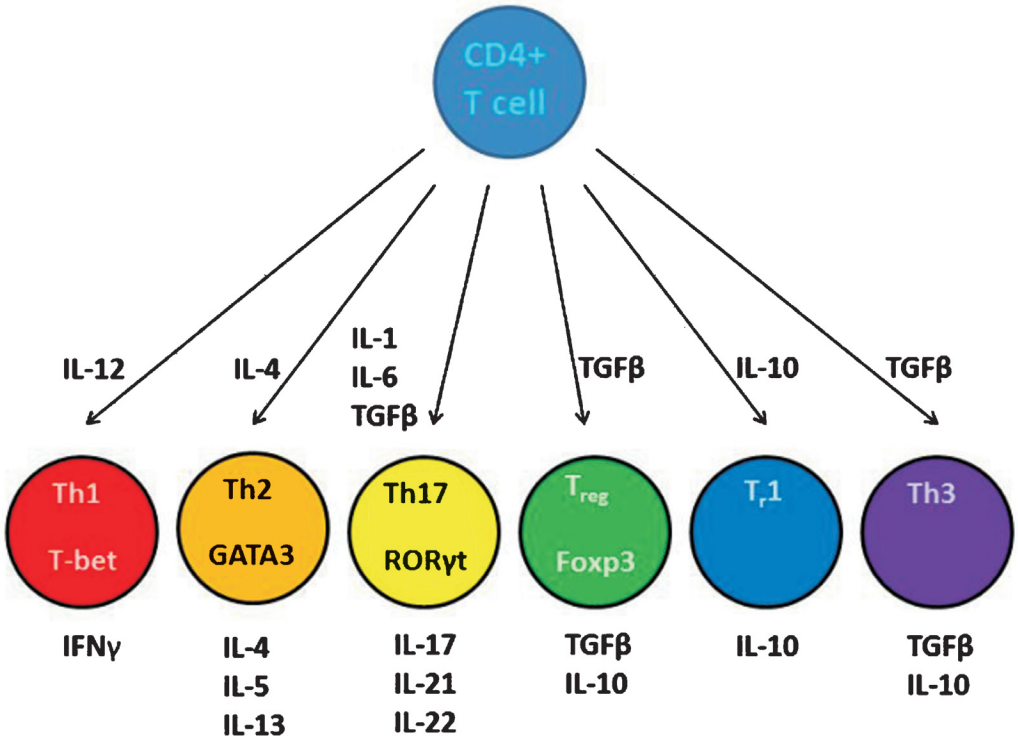

Fig. 1. CD4+ T cell differentiation and cellular phenotype. Naïve CD4+ T cells can differentiate into several different types of T helper (Th) or regulatory T (Treg or Tr) cells depending on the cytokine environment in which these are activated. The regulating transcription factors of these subsets and their characteristic effector cytokines are shown. (CD, cluster of differentiation; GATA3, GATA binding protein 3; T-bet, T-box expressed in T cells; RORγt, retinoic acid receptor-related orphan receptor gamma; Foxp3, Forkhead box p3; IL, interleukin; IFN, interferon; TGF, transforming growth factor.

produced by dendritic cells (DC) during an immune response are among the important factors that dictate the differentiation of T helper progenitor cells into specific subsets[32] (Fig. 1). For example, when activated DCs and macrophages produce interleukin (IL)-12, it activates the 'signal transducer and activator of transcription' (STAT) 4 pathway leading to the upregulation of the transcription factor 'T-box expressed in T cells' (T-bet). T-bet induces Th1 cell differentiation resulting in upregulation of interferon (IFN)γ and downregulation of 'GATA binding protein 3' (GATA3) and IL-4[32,33]. The Th1 cell produces IFNγ and promotes cellular immune response. In contrast, IL-4 signaling through STAT6 leads to a Th2 differentiation, upregulation of GATA3, and downregulation of IFNγ[34].

The Th2 cells produce IL-4, IL-5, and IL-13 and lead to B cell proliferation and antibody production. In this scheme of the two polar types of Th cells, Th1 and Th2, the immune responses and the diseases associated with them could be categorized as predominantly Th1- or Th2-mediated diseases. With the ability of Th1 to regulate Th2, and vice versa, the Th1-Th2 paradigm provided a fine conceptual framework to comprehend immune responses during health and disease. However, this Th1-Th2 paradigm had to be expanded and revised with the discovery of IL-23, which shares the p40 subunit with IL-12, and thereby was responsible for many of the immune effects that had previously been attributed to IL-12, or the lack of it[35]. In addition, newer cytokines (*e.g.* IL-17, IL-21, and IL-22) associated with inflammation were reported, which helped envision additional families of Th cells such as Th17 and Th22[29]. It has now been shown that Th17 cells are involved in many autoimmune diseases once thought to be primarily Th1-driven, including RA[36]. The Th17 cells produce IL-17, IL-21, IL-22, and IL-23, and play a role in RA and mucosal immunity[37]. In mice, the Th17 cells differentiate from naïve T cells in the presence of transforming growth factor (TGF) β and IL-6. This process involves signaling through STAT3 and increased expression of the transcription factor

'retinoic acid receptor-related orphan receptor gamma t' (RORγt)[38]. Alternatively, Th17 cells can differentiate in the presence of IL-21 and TGFβ, as in the case of deficiency of IL-6. However, in the absence of IL-6, T cells preferentially differentiate into Forkhead box p 3 (Foxp3)-expressing CD4+CD25+ Foxp3+ T_{Reg} cells, indicating that IL-6 is a regulator of the balance between Th17- T_{Reg}[39].

Regulatory T cells: There are several types of regulatory T cells. One of the recent additions to this group is the T_{Reg} cell. The phenotype of the T_{Reg} is CD4+CD25+Foxp3+. These cells can either differentiate naturally in the thymus (nT_{Reg}) or be induced in the periphery (iT_{Reg}). The differentiation of T_{Reg} requires TGFβ[40]. T_{Reg} cells suppress effector cells by producing TGFβ and IL-10 and via cytotoxic T-lymphocyte antigen (CTLA)-4 expressed on their cell surface. A subset of CD8+CD25+ Foxp3+ T_{Reg} has also been described[41]. Other regulatory subsets are T_r1 and T_h3[42]. The T_r1 cell is a CD4+CD25+Foxp3- T cell that requires IL-10 for its differentiation, and it secrets IL-10. The Th3 cell is a regulatory cell associated with the gut mucosa. It was shown to mediate the immunosuppressive effects of tolerance to the antigens administered via oral route (oral tolerance). Th3 cells mediate their suppressive action via secretion of TGFβ[42]. The balance between the effector T cells and the regulatory T cells determines whether or not autoreactive cells can induce an autoimmune response.

The effector functions of the cytokines that play a key role in arthritis pathogenesis

In RA and animal models of arthritis, inflammatory cytokines play a pivotal role in driving the disease process (Table I). Accordingly, biologic medications targeting these cytokines are being used for the treatment of arthritis. The cytokines that have been studied extensively are TNFα, IL-1, and IL-6[43]. TNFα has been at the center of RA research for several years because of its vital role in joint destruction and the control over other proinflammatory cytokines. It has been shown that TNFα controls the production of IL-1β and IL-8 by synovial cells[46]. In addition to increasing the release of other cytokines, TNFα can increase cellular infiltration into the synovium by enhancing chemokine expression, endothelial cell activation, and angiogenesis[43]. Finally, TNFα and IL-1 cause bone damage, the hallmark of RA pathogenesis[47,48]. In animal models of arthritis, IL-1β works in conjunction with IL-6 during the early phases of the disease, acting on endothelial cells to secrete chemokines like IL-8 and monocyte chemotactic protein (MCP)-1 to attract monocytes. IL-6 further upregulates chemokines that attract T cells, leading to enhanced cellular infiltration and beginning the transition from an acute inflammatory disease to a chronic immune disease[49]. These two phases are dominated by innate and adaptive immunity, respectively.

IL-17 is involved in the inflammatory response and it has been implicated in the pathogenesis of several autoimmune diseases including RA[50] and multiple sclerosis[51]. IL-17 is predominantly produced by the CD4+ Th17 cells. Other sources of IL-17 include CD8+ T cells, γδ T cells, natural killer T (NKT) cells, and lymphoid tissue inducer (LTi) cells[52,53]. There are six isoforms of IL-17, with IL-17A most commonly referred to as IL-17. IL-17 is a proinflammatory cytokine that plays an important role in inflammation of the synovium during RA. IL-17 can help promote the production of inflammatory cytokines like IL-6 and leukemia inhibitory factor (LIF)[50], and matrix degrading enzymes, matrix metalloproteinase (MMP) 1 and 3 by synovial fibroblasts[54]. IL-17 also induces osteoclastogenesis by upregulating receptor activator of NFκB (RANK) on osteoclast precursors and its ligand RANKL on the surface of activated T cells, resulting in bone erosion[55]. IL-17 also facilitates cellular infiltration either directly or by increasing the expression of chemokine (C-C motif) ligand (CCL) 20, CXC motif chemokine ligand (CXCL) 12, and CXCL5, and attracting B cells, T cells, neutrophils, and monocytes to the synovium[56-58]. The pannus formation (cellular infiltration into hyperplastic vascularized synovium) in an arthritic joint is enhanced further by the induction of IL-17-induced angiogenesis[59].

IL-17 can be regulated by other cytokines. IL-1β, TNFα, and IL-23 increase IL-17 expression[29,35-37], while IFNγ decreases IL-17 expression in autoimmune arthritis models[27]. IFNγ has for years been invoked as one of the major proinflammatory cytokines contributing to the pathogenesis of autoimmune diseases until the discovery of IL-17. However, recent studies have unraveled an opposite, anti-inflammatory role of IFNγ in arthritis models[27]. IFNγ-deficient mice showed an exacerbation of autoimmune arthritis and an increase in IL-17[60]. Similarly, we observed in the adjuvant arthritis model that IFNγ was expressed at the highest levels in the recovery phase of the disease[22]. Further, the treatment of rats either during the incubation phase or after disease onset with exogenous IFNγ reduced disease severity[27]. In addition, pre-treatment of rats with a C-terminal epitope 465-479 of rat heat-shock protein 65 (Rhsp65) suppressed arthritis, increased IFNγ production, but reduced IL-17 expression[22].

Table I. Cytokines in autoimmune arthritis

Cytokine	Cellular source	Receptors	Cells affected	Mechanism of action
TNFα	T cells, B cells, Mesenchymal cells, Monocytes	TNFRI (Inflammatory) TNFRII (Apoptotic)	Neutrophils, NK cells, T cells, Fibroblasts, Macrophage	Drives osteoclastogenesis, Inhibits collagen synthesis, Activates endothelium, Stimulates inflammatory cytokines and chemokines
IL-1	Monocytes, Macrophage	IL-1R1, IL-1R2 (Non-signaling), IL-1ra (natural antagonist)	T cells, Fibroblasts, Macrophage	Drives osteoclastogenesis, Upregulates MMPs, Drives angiogenesis, Stimulates inflammatory cytokines and chemokines
IL-6	T cells, B cells, Mesenchymal cells, Macrophage, Endothelial cells	gp130 and IL-6R	T cells, B cells Macrophage, Chondrocytes	Autoantibody production, Induces acute phase protein, Stimulates bone resorption,
IFNγ	T cells, NK cells, B cells, NKT cells, Macrophage	IFNGR1 and IFNGR2	T cells, B cells Macrophage,	Stimulates Th1, Inhibits Th17, Antigen presenting cell activation (MHCII, CD80), Inhibits osteoclastogenesis, Inhibits neutrophil trafficking

TNF-α, Tumour necrosis factor-alpha; TNFR, TNF receptor; NK, natural killer; NKT, natural killer T; IL, interleukin; IL-R, interleukin receptor; IFN, interferon; IFNGR, interferon gamma receptor; MMPs, matrix metalloproteinases; MHC, major histocompatibility complex

Source: Refs 29, 43-46

IFNγ functions as a regulatory cytokine, directly as well as indirectly, in autoimmune arthritis. One indirect effect that IFNγ has is the upregulation of IL-27, a cytokine that can reduce IL-17 production. IL-27 is an IL-12 superfamily cytokine, and it can prevent the differentiation of Th17 by reducing both the expression of RORγt and the phosphorylation of STAT3. Both of these transcription factors are integral to the differentiation of Th17[27]. IL-27 is comprised of two subunits, Epstein-Barr virus-induced gene 3 (EBI3) and p28. It is secreted by macrophages, dendritic cells, epithelial cells and a wide range of innate and adaptive immune cells, most notably the CD4+ T cells[61]. In addition to preventing pathogenic T cell differentiation, IL-27 can induce both the expression of program death-ligand 1 (PD-L1), a co-receptor that is a negative regulator of T cell function[56], and the generation of IL-10-producing Tr1 cells[62]. IL-27 is also able to downregulate the expression of RANK on osteoclast precursors and RANKL on CD14+ cells, causing a decrease in osteoclastogenesis and thereby limiting bone loss[63]. Though the role of IL-27 in autoimmunity has not yet been fully defined, increasing evidence points to its anti-inflammatory and anti-arthritic activities.

Kinetics of cytokine expression during the course of arthritis and its correlation with disease susceptibility versus resistance

Animal models of human RA[29] permit comprehensive experimental studies on the pathogenesis of autoimmunity, including the role of T cells and cytokines in the disease process. Two of the commonly used models of RA are adjuvant arthritis (AA) in rats[7] and collagen-induced arthritis (CIA) in mice/rats[64]. The cytokines released during the course of autoimmune arthritis influence the severity of the pathological and clinical features of the disease (Figs 1 and 2). The AA model system has extensively been used to examine the disease-related events at different time points in the course of the disease as well as for testing potential anti-arthritic agents for their therapeutic efficacy and side effects. However, not all rat strains are equally susceptible to autoimmune arthritis. The Lewis rat is highly susceptible to AA, whereas the Wistar-Kyoto (WKY) rat is resistant to arthritis despite having a similar major histocompatibility complex (MHC)

haplotype as the Lewis rat[5,11,21]. WKY rats provide a good control for Lewis rats for studies on disease pathogenesis. In our studies, we have exploited this pair of rat strains for addressing important aspects of AA, for example, epitope mapping of the disease-related antigen Bhsp65[5,11], cellular migration into the joints[67], the dynamics and epitope reactivity of antibodies produced during AA[68], and cytokine responses against Bhsp65[22,23,27,69].

In different studies performed in the AA model, cytokine responses have been examined in the draining lymph nodes, spleen, synovial-infiltrating cells (SIC), or joint homogenates. Also, not all time points have been tested in each tissue. This makes it somewhat difficult to directly compare the profiles obtained using one tissue with that derived from another tissue. However, it is understandable that the peripheral lymphoid tissues and SIC might show similarities in certain cytokine responses, but differences in others. A set of representative profiles of pro-/anti-inflammatory cytokines in AA constructed from the results of different studies[22,23,27,65,66] is shown in Fig. 2. In rats with AA, the expression of pro-inflammatory cytokines (TNFα, IL-1 and IL-6) showed a gradual increase from the incubation phase to the 'onset' phase. After that point, IL-1 and IL-6, but not TNFα levels decreased during the regression phase. Surprisingly, IL-17 expression was highest in the incubation phase of the disease, but thereafter its levels remained low throughout the disease. In contrast, the expression of IFNγ, IL-27, and IL-10 was low during the early incubation and onset phases of the disease, but it increased during the late stages correlating with disease regression. IL-10 is a well known anti-inflammatory cytokine. Our results point towards a disease-regulating role of IFNγ and IL-27 in AA. IFNγ is considered to be a prototypic pro-inflammatory cytokine. However, our studies have shown that this cytokine also possesses arthritis-suppressive activity[22]. In the case of IL-27, which is among the newer cytokines whose functional attributes have not yet been fully defined, our results[27] clearly demonstrate that it has anti-arthritic activity. For this reason, we have depicted IFNγ, IL-27, and IL-10 under anti-inflammatory cytokines (Fig. 2).

In contrast to the AA-susceptible Lewis rats, the AA-resistant WKY rats had a different cytokine profile that helped us comprehend the lack of signs of disease following an arthritogenic challenge[27]. The pattern of expression of IL-17 in WKY rats was similar to that of Lewis rats. However, unlike Lewis rats, WKY rats also showed highest levels of expression of IL-27 and IFNγ at the same time as that of IL-17. We suggested that the concurrent expression of these two cytokines with IL-17 helped neutralize the pathological effects

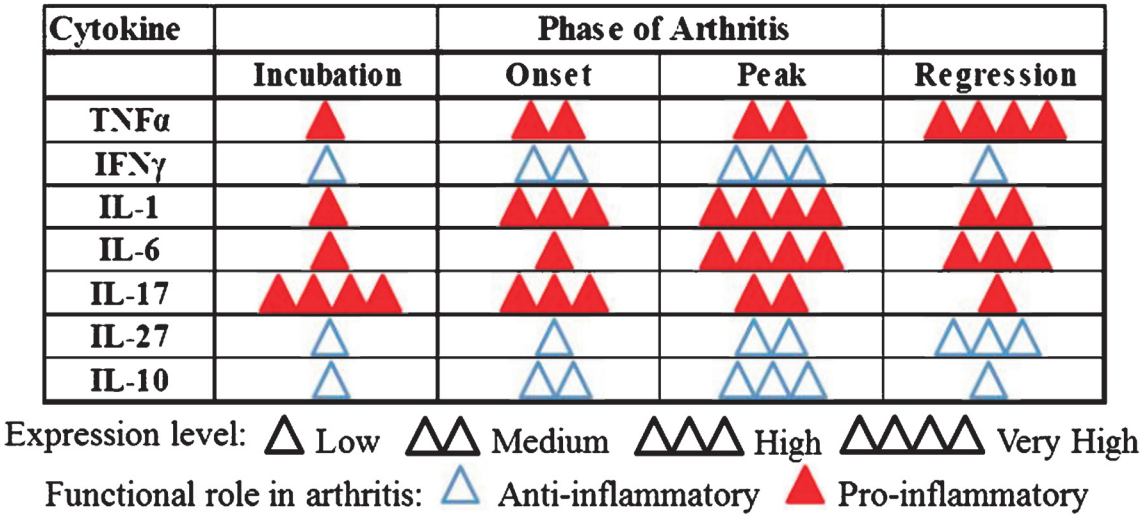

Fig. 2. The dynamics of cytokine expression during the course of adjuvant arthritis. Adjuvant arthritis (AA) in the Lewis rat, induced following immunization with heat-killed *M. tuberculosis* H37Ra, displays distinct phases of the disease. These phases include incubation, onset, peak and regression. Proinflammatory cytokines play a vital role in the initiation and progression of arthritis, whereas anti-inflammatory cytokines facilitate regression of inflammatory arthritis. The levels of cytokines represented by the number of triangles are relative to each phase for that particular cytokine. (IL, interleukin; IFN, interferon; TNF, tumour necrosis factor).
Source: Refs 22, 23, 27, 65, 66

of IL-17 in WKY rats[27]. In contrast, IL-17 activity was unopposed in the incubation period in the case of Lewis rats, thereby explaining the development of arthritis.

Our results of modulation of AA by treatment of Lewis rats with exogenous cytokine yielded interesting results[27]. The treatment of Lewis rats with IL-17 in the incubation phase of the disease leads to disease aggravation as expected from the pathogenic role of IL-17 in AA. The most interesting finding was the effect of IL-27 treatment on the inhibition of AA. These results demonstrate that IL-27 plays an immunoregulatory role in AA[27]. Surprisingly, the treatment of rats with IFNγ or TNFα also offered protection against AA, showing that the timing of administration of certain cytokines that are typically considered to be proinflammatory (*e.g.* IFNγ and TNFα) may also lead to the suppression of arthritis[22,23,27]. This emphasizes the dual role of these proinflammatory cytokines[69].

Taking together, studies on the temporal expression of cytokines in the AA-susceptible/-resistant rats have provided important and useful information regarding arthritis development and its deliberate control by cytokine treatment. For example, the cytokine profiles have provided insights into the relative contribution of different cytokines in the initiation and progression of AA, followed by the regression of inflammation (Fig. 2). Further, comparative studies in Lewis/WKY and other AA-susceptible/resistant rodent strains have offered interesting insights into differential cytokine responses in the face of comparable T cell proliferative response to the disease-related antigens. In addition, such studies have underscored the significance of the balance between the pro- and anti-inflammatory cytokines in determining (in part) whether arthritic inflammation would result or not following an arthritogenic stimulus, such as Mtb injection. Finally, the study of the cytokine kinetics has also permitted validation of the disease-protective versus disease-aggravating effects of specific cytokines as tested by the treatment of rats/mice at different phases of the disease.

Cellular migration into the joints and characteristics of the synovial-infiltrating cells

Cellular infiltration into the synovium is a characteristic feature of RA. The resulting pannus invades the surrounding bone and cartilage and leads to tissue damage, pain and disability in the affected joints. There are various cell types that infiltrate the synovium of arthritic joints. The T cell migration into the synovium plays an important role in the activation and recruitment of other cell types. Most notably, Th17 cells that migrate into the synovium lead to neutrophil recruitment via IL-17-mediated induction of chemokines[70]. It has recently been shown that IL-17-induced neutrophil recruitment occurs indirectly through TNFα, which binds its receptor TNF receptor (TNFR)1[71]. T_{reg} cells have also been isolated from inflamed synovium[71], but at present the relative kinetics and frequencies of Th17 and Treg in the joints during the course of arthritis in animal models or RA patients are not yet defined.

Other cell types that are found in arthritic joints are macrophage- and fibroblast-synoviocytes, neutrophils, B cells, dendritic cells, and mast cells[72]. This cellular migration followed by accumulation of cells in the joints correlates with arthritis susceptibility as shown in our comparative study in the AA model using arthritis-susceptible Lewis rats and arthritis-resistant WKY rats[67]. Chemokines play an important role in cellular migration into the joints. Chemokines are upregulated by cytokines and can reciprocate, and lead to the increased cytokine expression causing persistent inflammation[73]. As a result, chemokines can be targeted directly or indirectly through the suppression of cytokines to treat RA. We have recently shown that a natural plant product (celastrol) can decrease inflammatory cytokine and chemokine production leading to the inhibition of cell migration and suppression of arthritis in the AA model[74].

Angiogenesis and its role in the pathogenesis of arthritis

Angiogenesis refers to the formation of new blood vessels from existing vessels, and it facilitates the nourishment and maintenance of growing tissue. Angiogenesis is considered to be one of the key mechanisms that promote chronic joint inflammation in RA[75]. During RA, hyperplastic synovium with immune cell infiltrates forms the pannus, which requires supply of oxygen and nutrients. Angiogenesis contributes to the formation and maintenance of the pannus in RA. The pannus is highly vascularized and it invades the articular cartilage and bone[76]. The actions of cytokines and other mediators of inflammation produced by the synovial-infiltrating cells result in cartilage damage and bone erosion in arthritic joints[76,77]. Inhibition of angiogenesis by interfering with pathways driven by the vascular endothelial growth factor (VEGF) or other mediators results in delayed onset and reduced severity of arthritis in animal models, such as CIA in mice and

AA in rats[78-82]. These observations provide additional support for the role of angiogenesis in the initiation and propagation of RA.

Angiogenesis involves multiple cell types and complex interplay of mediators and pathways (Table II). The formation of new vessels from a pre-existing vessel is a result of a series of events including selective degradation of vascular basement membrane and adjacent extracellular matrix, and migration of endothelial cells leading to tube formation and capillary growth[90,91]. VEGF is a key factor mediating angiogenesis. The levels of VEGF in the serum and synovial fluid correlate with the severity of RA. High requirement of oxygen in the pannus, the growing tissue in arthritic joints, makes that tissue relatively hypoxic. Hypoxia induces gene expression of pro-angiogenic proteins such as VEGF via the activation of transcription factors known as hypoxia-inducible factors (HIFs)[91]. Activation of HIFs can also occur by proinflammatory cytokines. VEGF in turn recruits monocytes, which differentiate into macrophages and secrete matrix-degrading enzymes known as matrix metalloproteinases (MMPs). VEGF also induces the migration and proliferation of endothelial cells and smooth muscle cells, which lead to the formation of new capillaries[78,83,92]. Epidermal growth factor (EGF), fibroblast growth factor-2 (FGF-2), hepatocyte growth factor (HGF), insulin-like growth factor (IGF), and platelet-derived growth factor (PDGF) serve as additional mediators of angiogenesis in RA[25,92,93] (Table II).

Several cytokines such as TGF-β, TNF-α, IL-1, IL-6, IL-8, IL-13, IL-15, and IL-18 modulate the process of angiogenesis[84,94-102] (Table III). For example, TGF-β and IL-1 stimulate the secretion of VEGF through the activation of HIF. These events are mediated via mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathway. The above-mentioned cytokines further enhance VEGF secretion induced by hypoxia. This cooperation between cytokines and hypoxia facilitates the secretion of large amounts of VEGF by synovial cells in the hypoxic environment in the arthritic joint resulting in increased angiogenesis.

A variety of chemokines contribute to the angiogenic process in RA (Table II). The expression of CXCL12 [or, stromal cell-derived factor 1 (SDF-1)], is influenced by cytokines and hypoxia. SDF-1 in turn facilitates the recruitment of lymphocytes into

Table II. Mediators and inhibitors of angiogenesis

Mediators of angiogenesis	Endogenous inhibitors of angiogenesis	Examples of angiogenesis inhibitors being tested for anti-arthritic activity
Growth factors and receptors: VEGF, VEGF receptors (Flk-1 and Flt-1), HIFs, PDGF, FGF-2, EGF, IGF, HGF Cytokines: TNF-α, IL-1, IL-6, IL-17, IL-18, IL-23, TGF-β, IL-13, IL-15 Chemokines: IL-8, CX_3CL1 (Fractalkine), CCL21 (Exodus-2) and CXCL12 (SDF-1) Others: MMPs, angiogenin, PAF, angiopoietin, adhesion molecules, MIF, JAMs, FAKs, SAA, sphingosine kinase	Angiostatin, endostatin, Thrombospondin-1 and -2, IL-4	Soluble VEGF receptor (Flt), Paclitaxel, 2-methoxyestradiol, fumagillin analogues (TNP-470, PPI-2458), anti-integrin αVβ3 antibody (LM-609), PTK-787, Bevacizumab (anti-VEGF antibody), thalidomide.

VEGF, vascular endothelial growth factor; HIFs, hypoxia-inducible factors; PDGF, platelet-derived growth factor; FGF-2, fibroblast growth factor-2; EGF, epidermal growth factor; IGF, insulin-like growth factor; HGF, hepatocyte growth factor; TGF-β, transforming growth factor-beta; TNF-α, tumour necrosis factor-alpha; CX_3CL1, C-X_3-C motif chemokine ligand 1; CCL21, C-C motif chemokine ligand 21; CXCL12, C-X-C motif chemokine ligand 12; SDF-1, stromal cell-derived factor 1; MMPs, matrix-metalloproteinases; PAF, platelet activating factor; MIF, macrophage migration inhibitory factor; JAMs, junctional adhesion molecules; FAKs, focal adhesion kinases; SAA, serum amyloid A

Source: Refs 24, 25, 78, 83-89

Table III. MMPs, ADAMTs and cathepsins, and their target molecules

Regulator(s)	Proteinases	Targets
IL-1β, TNF-α and IL-17 produced in arthritic joints	MMP-1, -8 and -13	Collagen I, II and III
	MMP-2 and -3	Collagen IX and XI
	MMP-2 and -9	Denatured collagen (gelatin)
	MMPs and ADAMTS	Aggrecans
	ADAMTs	Proteoglycans
	Cathepsins (B, L, K)	Collagen I and II, and Proteoglycans

IL, interleukin; TNF-α, tumor necrosis factor-alpha; MMPs, matrix metalloproteinases; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs species.
Source: Refs 96-102

the arthritic synovium[85]. The chemokine CX_3CL1 (or, fractalkine), also induces angiogenesis[103]. CCL21 (Exodus-2) induces endothelial cell migration and tube formation. These events involve PI3K[104].

Other mediators that influence angiogenesis include angiogenin, platelet activating factor (PAF), angiopoietin, soluble adhesion molecules, and endothelial mediator endoglin. Further, macrophage migration inhibitory factor (MIF), junctional adhesion molecules (JAMs) and focal adhesion kinases (FAKs) are associated with inflammatory angiogenesis. Serum amyloid A (SAA) and sphingosine kinase have also been invoked in the angiogenic events[25] (Table II).

Matrix metalloproteinases (MMPs) and other cartilage-degrading enzymes

Articular cartilage, which is made of extracellular matrix (ECM) and chondrocytes, is the major tissue targeted in the joints in RA. In uncontrolled RA, there is a progressive damage to the joints leading to physical disabilities. Cartilage ECM contains primarily proteoglycans, aggrecan and collagen. Aggrecan associates with proteoglycans to form a network of collagen fibers, which are the main structural component of the matrix[105]. Articular cartilage is mainly composed of type II collagen, but it also contains collagen type IX XI and VI. The cartilage matrix also contains leucine-rich proteoglycans, including decorin, fibromodulin and biglycan[105]. Cartilage degradation can be mediated by a variety of proteases including MMPs, cathepsins (B, L, K) and "a disintegrin and metalloproteinase with thrombospondin motifs species" (ADAMTS)[96-102] (Table III).

Cartilage degradation involves depletion of aggrecan and breakdown of collagen fibers[100]. Inflammatory immune cells in the joints release proinflammatory cytokines such as IL-1β, TNF-α and IL-17. These cytokines induce expression of MMPs (MMP-1, -2, -3, -7, -8, -9 and -13) and ADAMTS (ADAMTS 1, 4 and 5) in the synovial fibroblasts and chondrocytes[106-109]. Proinflammatory cytokines are the main inducers of MMPs in these cells[101]. In addition, there is endogenous regulation of these enzymes in synovial fibroblasts. Members of both the MMP and ADAMTS families contribute to aggrecan degradation. Collagen type II resists degradation by most proteases owing to its triple-helical structure. Only MMP-1, -8, and -13 can degrade fibrillar collagens including types I, II and III collagen[110]. Following cleavage of the collagen molecules, the helices are disrupted and collagen is denatured into gelatin. Gelatin then forms the substrate for gelatinases, namely MMP-2 and -9[96,97,110]. Type IX and XI collagens are degraded by MMP-3 and MMP-2[102]. Other proteinases (cathepsins and ADAMTS) also play a role in the tissue damage in the joints[96-99,101,111]. Inhibition of these enzymes is expected to offer protection against cartilage destruction in RA. Several therapeutic interventions to treat arthritis in animal models have shown to inhibit the activities of MMPs, particularly MMP-9. For example, the treatment of arthritic rats with IL-27 or celastrol has a significant inhibitory effect on MMP-9 activity[27,28]. (Celastrol is a bioactive component of a Chinese herb, Celastrus and other related plants). In addition, selective MMP inhibitors have been tested for their cartilage-protective effects in experimental models of arthritis[112]. However, there is not much information available on the inhibition of ADAMTS in

RA. The development of effective and safe inhibitors of MMPs and ADAMTS would offer new therapeutic agents to limit the joint destruction.

Tissue inhibitors of metalloproteinases (TIMPs) were originally identified as endogenous proteins that regulate MMPs. However, TIMPs also can regulate ADAMTS[113]. Four members of the TIMPs family, namely TIMP1, 2, 3 and 4 have been identified. The expression of TIMPs is tissue specific[113,114]. At present, there is not much information on the role of TIMPs in RA. TIMPs are also known to influence cell growth and differentiation, cell migration, and angiogenesis, and these effects are independent of their inhibitory effect on MMPs[115]. Imbalance in the ratio of MMPs to TIMPs promotes abnormal degradation of ECM[116]. Therapeutic interventions to treat arthritis in animal models have been shown to maintain the balance between MMPs and TIMPs[117]. For example, Sinomenine, an alkaloid derived from the Chinese medicinal plant, *Sinomenium acutum,* has been shown to ameliorate CIA in rats by maintaining the balance between MMPs and TIMPs[117]. However, the protective role of TIMPs in RA has yet to be further explored.

Influence of vascular permeability and vascular endothelial cell characteristics on target organ-directed autoimmunity

Recent studies have highlighted the role of vascular permeability of the blood vessels in the joints[4] and that of the fine characteristics of the vascular endothelial cells of the joint vasculature[26] in preferentially directing the systemic autoimmune responses to the joints. The K/BXN model of arthritis represents an autoimmune response against a systemic antigen, GPI. One of the mechanisms proposed for the preferential targeting of the distal joints of the paws invoked a vascular leak[4]. In our study on the AA model in which ubiquitously distributed Bhsp65 has been implicated in arthritis pathogenesis, we identified phage-encoded peptides that preferentially homed to the arthritic joints and showed binding to CD31+ vascular endothelial cells in the inflamed joints[26]. One of the two peptides also possessed anti-arthritic activity in addition to joint-homing attributes. Similarly, another study conducted in immunodeficient mice engrafted with human synovial tissue also reported isolation of synovial-binding peptides selected after the phage screening[118]. Taken together, these studies have opened up new avenues for further exploration of the characteristics of the joint vasculature in rendering the joints preferential targets of autoimmune attack. Additional studies are needed to determine the functional significance of unique attributes of the blood vessels as well as vascular endothelial cells in arthritis besides the much appreciated role of angiogenesis in this disease.

Therapeutic approaches for the control of autoimmune arthritis

RA is an autoimmune disease driven by proinflammatory cytokines. Therefore, if the proinflammatory cytokines can be reduced, the inflammatory component of the disease can be suppressed and the destruction of bone and cartilage limited. Several disease-modifying antirheumatic drugs (DMARDs) are now available for the management of RA patients. These medications target inflammatory mediators and are less toxic compared to other drugs for RA. One of the newer groups of anti-arthritic drugs is biologics, which are based on inhibiting the actions of proinflammatory cytokines. The first group of these drugs targets TNFα- infliximab (a chimeric monoclonal antibody), etanercept (TNFR-Fc) and adalimumab (a human monoclonal antibody)[119,120]. The anti-TNFα therapy has worked well, but approximately 40 per cent of RA patients fail to respond to this treatment[120,121]. Accordingly, there are drugs targeting IL-1- Anakinra (recombinant IL-1 receptor antagonist) and AMG 108 (human monoclonal antibody against IL-1β), or IL-6 receptor - Tocilizumab (a human monoclonal antibody against IL-6 receptor). There was excitement with the targeting of IL-1 because IL-1 was shown to be solely responsible for cartilage damage but partially responsible for bone damage in mice transgenic for TNFα but deficient in IL-1[122]. The IL-1-directed drugs have shown some efficacy in limiting inflammation, but IL-1 inhibition with Anakinra proved to have less bone damage protection than that observed with anti-TNFα treatment[123].

As research on newer cytokines expands, there are increasing opportunities for new drugs to treat RA. IL-17 plays a critical role in the induction of arthritis[124]. Research on IL-17 has led to the development of a humanized anti-IL17 monoclonal antibody LY2439821, which has gone through phase-I clinical trials to determine patient tolerability and efficacy[125]. This anti-IL-17 antibody added to other DMARDs improved signs and symptoms of RA patients[125]. Since many cytokines signal through the Janus Kinase- Signal Transducer and Activator of Transcription (JAK-STAT) pathway, some new drugs targeting this pathway have been explored as therapeutics. For example, Tofacitinib (a JAK1 and JAK3 inhibitor), showed inhibition of the

key cytokines relevant for arthritis, such as IL-17, IL-6 and IL-8[126].

Angiogenesis plays an important role in the disease pathogenesis in arthritis. Several endogenous factors that have angiostatic activity are produced by the synovium of arthritic joints[93], but these factors may fail to effectively control angiogenesis and inflammation associated with arthritis. In this regard, several agents/approaches including some endogenous metabolites are being examined to control angiogenesis for eventual therapeutic use[24,86-89] (Table II).

Finally, complementary and alternative medicine (CAM) products are increasingly being used by arthritis patients, who are seeking alternatives to expensive and toxic conventionally used drugs. Several studies have highlighted the anti-arthritic activity of various natural plant products in experimental models of arthritis[127,128]. For example, in studies in the AA model, extracts of green tea[129], celastrus[28,30,130], and huo-luo-xiao-ling dan[131,132] have been shown to offer protection against arthritis. We hope that a systematic validation of herbal products in RA patients might offer promising adjuncts to conventional drugs for the management of this debilitating disease.

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