

Review

Recent developments in the immunobiology of rheumatoid arthritis

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Abstract

Progress into the understanding of immunopathology in rheumatoid arthritis is reviewed in the present article with regard to pro-inflammatory cytokine production, cell activation and recruitment, and osteoclastogenesis. Studies highlight the potential importance of T helper 17 cells and regulatory T cells in driving and suppressing inflammation in rheumatoid arthritis, respectively, and highlight other potential T-cell therapeutic targets. The genetic associations of the HLA shared epitope alleles with antibodies to citrullinated peptides in rheumatoid arthritis patients indicate that T cells are providing help to B cells to produce autoantibodies, and there is increasing evidence that these autoantibodies are pathogenic in rheumatoid arthritis.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterised by chronic inflammation of the joint. Although the precise pathogenesis of RA remains unclear, T cells, B cells, macrophages, neutrophils and synovial fibroblasts are central to the mechanisms of joint inflammation and disease progression. The genetic association of HLA-DR1 and HLA-DR4 with RA suggests that the disease is at least partially driven by T cells. The role of T cells has not, however, been conclusively demonstrated in the pathogenesis of RA – although the success of abatacept (CTLA-4lg) in clinical trials [1] implies that rheumatoid T cells are important in driving the inflammatory process, and thus T cells could be targeted in clinical therapy.

The role of B cells in RA pathology has been highlighted by the clinical improvements in RA patients receiving B-cell-depleting therapies such as rituximab, an anti-CD20 antibody [2], and the increased interest in the role of autoantibodies in RA. In addition to producing antibodies, proinflammatory cytokines and chemokines, B cells efficiently act as antigen-presenting cells themselves and thus influence T-cell activation and expansion [3,4].

In the present review we look at recent developments in the immunobiology of RA, with focus on the role of T cells and B cells, the products they produce, including cytokines and autoantibodies, and the genetic factors potentially involved in their regulation and function.

T cells

In contrast to the clearly defined role of macrophage-derived cytokines such as TNF α in the pathogenesis of RA, the relevance and contribution of the T cells is not clear and has been challenged [5]. In particular, the expectation that the increased T cells in the synovium are a result of clonal expansion to a given antigen has not been established. An HLA-restricted T-cell response to antigen is suggested, since over 80% of Caucasian RA sufferers have a shared epitope (SE) conserved across the HLA-DR1 and HLA-DR4 haplotypes (0101, 0401, 0404 and 1402) [6]. No overall consensus has been reached, however, on the potential autoantigens involved. T-cell responses to collagen type II, heat shock proteins and microbial antigens have been reported in a small proportion of RA patients (reviewed in [7]), and more recently autoantibodies to deiminated 'citrullinated' peptides have been described, suggesting that they may be important autoantigens in this disease. This aside, the concordance for disease in identical twins is still less than 15%, suggesting other factors are of major importance.

Rheumatoid T cells have an unusual phenotype. While these cells maintain a highly activated phenotype indicated by high expression of CD69, transferrin receptor and HLA-DR, they are nonetheless hyporesponsive to antigenic stimulation [8-10]. Brennan and colleagues demonstrated that the spontaneous TNF α production in RA synovium was largely T-cell dependent [11], suggesting that regulation of T-cell function might be important to control the disease. It is thus not

CCP = cyclic citrullinated peptide; CIA = collagen-induced arthritis; FoxP3 = forkhead box P3; IFN = interferon; IL = interleukin; PTPN22 = protein tyrosine phosphatase N22; RA = rheumatoid arthritis; RF = rheumatoid factor; SE = shared epitope; SNP = single-nucleotide polymorphism; STAT4 = signal transducer and activator of transcription 4; TGF β = transforming growth factor beta; Th cells = T helper cells; TNF = tumour necrosis factor; TRAF-1 = TNF receptor-associated factor 1; Treg cells = regulatory T cells.

surprising that treatment with a nondepleting anti-CD4 antibody (keliximab) has some clinical efficacy in RA patients [12-14]. Owing to unacceptable side effects, however, anti-CD4 clinical trials were not pursued [15]. Clinical trials with abatacept, on the other hand, look more promising [1,16,17]. Abatacept inhibits activation of T cells by blocking the interaction between CD28 on T cells and B7 on antigen-presenting cells. In recent phase III clinical trials, abatacept showed a similar disease-modifying efficacy as infliximab treatment, the most successful treatment so far, in RA patients with an inadequate response to methotrexate [18]. Furthermore, abatacept has less adverse effect than infliximab, suggesting it is biologically safer and a more tolerated treatment [18,19].

In addition to blocking the interaction between T cells and antigen-presenting cells, there are several other targeting possibilities for T-cell-based intervention including prevention of T-cell infiltration, inhibition of T effector cell activation and induction of regulatory T cells.

Cellular trafficking and cross-talk

An extensive array of cytokines, chemokines and adhesion molecules has been detected in the synovium of patients with RA and considered of importance in the migration of cells to the synovium (reviewed in [20]). A recent study by Kop and colleagues show that neutralisation of CD97, a member of the epidermal growth factor seven-span transmembrane family of TM7 adhesion receptors, increases resistance to collagen-induced arthritis (CIA) in mice, indicating that interaction between CD97 and its ligands may be involved in cell migration in arthritis [21]. CD97 is expressed by inflammatory cells, mainly leukocytes, in RA synovium [22]; the ligands for CD97 (CD55, chondroitin sulphate B, and $\alpha_5\beta_1$) are also expressed in this tissue [22,23].

Although antigen-dependent T-cell responses may be important in initiating the inflammatory response during arthritis, there is evidence that antigen-independent responses also play a role in RA. The RA synovial T cells can activate human monocytes/macrophages in a contact-dependent manner to induce the expression of inflammatory cytokines, including TNF α [24,25]. A recent study further demonstrated that RA synovial T cells induce monocyte CC chemokine production (monocyte chemoattractant protein 1, macrophage inflammatory protein 1 alpha, macrophage inflammatory protein 1 beta and RANTES) and CXC chemokine production (IL-8, growth-related gene product alpha and IP-10) in a contact-dependent manner. This effector function was also shared by T cells activated with a cytokine cocktail (IL-2, IL-6 and TNF α) [26]. Furthermore, Tran and colleagues reported that T cells activated by IL-2, IL-6 and TNF α also induce fibroblast-like synoviocytes to produce inflammatory cytokines such as IL-6 and IL-8 in a cell contact-dependent manner [27]. These studies provide further evidence that T cells can be important drivers of chronic inflammation through antigen-independent mechanisms.

Cross-talk between natural killer cells and monocytes also results in the sustained stimulation of TNF α production. Natural killer cells activated by IL-15 activate monocytic cells to synthesise TNF α in a contact-dependent manner; in turn, monocytic cells induce CD69 expression and IFN γ production in natural killer cells, an effect mediated by β integrins and membrane-bound IL-15. IFN γ further increased production of membrane-bound IL-15 in monocytic cells, and neutralising membrane-bound IL-15 and β_2 integrins inhibited TNF α production – suggesting that membrane-bound IL-15 and β_2 integrins are important in the cross-talk between natural killer cells and monocytes [28]. The pathogenic role of IL-15 in RA has been confirmed in a phase I/II trial with anti-IL-15 therapy in RA patients [29].

T helper 17 cells

There is increasing evidence that IL-17 plays a role in the immunopathology of RA. This proinflammatory cytokine is produced by T helper (Th) 17 cells, which represent a recently discovered CD4⁺ effector T-cell lineage distinct from Th1 cells, Th2 cells and regulatory T (Treg) cells. IL-17 has pleiotropic effects on many cell types including macrophages, fibroblasts, epithelial cells, endothelial cells and mesenchymal cells, where it induces upregulation of nuclear factor kappa B and HLA class I as well as neutrophil chemokines and cytokines such as TNF α , IL-1 β , IL-6 and granulocyte-macrophage colony-stimulating factor [30-33]. Important in RA pathogenesis are the effects of IL-17 in driving osteoclastogenesis leading to bone resorption. All of these effects together lead to joint destructions and chronic inflammation [33,34]. Human RA cells expressing high levels of IL-17 are present in the synovium and circulation [34-40], and IL-17 mRNA levels in synovial membranes are predictive of joint damage progression in RA [41].

Recent work by Fath and colleagues suggests that CD4⁺CD28^{null} cells, if activated within the synovial membrane, may potentially act as a negative regulator for the differentiation of Th17 cells in RA. An increased frequency of CD4⁺ T cells lacking expression of CD28 has been reported in peripheral blood of RA patients (reviewed in [42]). Fath and colleagues more recently reported that CD4⁺CD28^{null} T cells were infrequent in synovial membrane and synovial fluid, despite significant frequencies in the circulation of RA patients [43]. It is interesting to note that CD4⁺CD28^{null} cells in synovial fluid were able to produce high levels of IFN γ upon antigenic stimulation. This T-cell-derived cytokine is rarely found in synovial membranes [44], and has been reported to block the differentiation of Th17 cells [45]. Nevertheless, the ability of CD4⁺CD28^{null} cells to regulate the differentiation of Th17 within rheumatoid synovium remains to be demonstrated.

A role for IL-17 in experimental arthritis has been demonstrated. CIA is suppressed in IL-17-deficient mice, and administration of neutralising anti-IL17 antibodies significantly reduces the severity of CIA (reviewed in [46]). Autoimmune

arthritis in SKG mice also appears to be highly dependent on the CD4⁺ T cells secreting IL-17 [47]. The SKG mouse strain spontaneously develops T-cell-mediated autoimmune arthritis, which clinically and immunologically resembles RA due to a mutation of the gene encoding ZAP-70, a key signal transduction molecule in T cells [48]. SKG mice develop thymus-produced self-reactive T cells that are constantly activated in the periphery, and that proliferate and differentiate to Th17 cells. *In vivo* development and expansion of Th17 cells, and consequently arthritis, were dependent on IL-6 produced by either T cells or non-T cells. IL-17 or IL-6 deficiency completely inhibited arthritis, whereas IFN γ deficiency exacerbated the disease [47]. A genetic polymorphism might therefore contribute to thymic generation of potentially arthritogenic self-reactive T cells, which form a cytokine milieu that facilitates differentiation into self-reactive Th17 cells.

Recent data, however, suggest that IL-17 production is regulated differently and has a somewhat different effect in humans compared with mice. While development of mouse Th17 cells require transforming growth factor beta (TGF β) plus IL-6, human Th17 cell development appears to be independent of these cytokines but requires IL-23 and IL-1 β [49-53].

T cells and osteoclastogenesis

T cells are important contributors to the pathogenesis of bone erosion in RA through induction of osteoclastogenesis. Miranda-Carus and colleagues recently showed that autologous T-cell monocyte cocultures derived from peripheral blood of patients with early RA, but not from healthy control individuals, resulted in osteoclast differentiation dependent on RANKL – which is expressed by activated T cells and RA synovial fibroblasts [54,55] – and augmented by IL-15, IL-17, TNF α and IL-1 β [56]. Other studies have identified Th17 cells as the exclusive osteoclastogenic T-cell subset among the CD4⁺ T-cell lineages [34,57].

Yago and colleagues further reported that recombinant human IL-23 was able to induce osteoclastogenesis in macrophage-colony-stimulating factor-differentiated human peripheral blood mononuclear cells, and the process was independent of RANKL but dependent on osteoprogesterin, IL-17 and TNF α . Furthermore, anti IL-23 treatment significantly improved inflammation and bone erosion in a CIA model in rat [58]. This further demonstrated a direct involvement of T cells in pathogenesis of RA.

T-cell activation and apoptosis

Regulation of T-cell apoptosis is critical for lymphocyte homeostasis and immune function. Inhibition of T-cell apoptosis in the synovium of patients with established RA was first described in 1995 [59]. Raza and colleagues recently showed that inhibition of synovial fluid leukocyte apoptosis in the earliest clinically apparent phase of RA distinguishes this from other early arthritides [60]. Patients

with early RA had significantly lower levels of neutrophil apoptosis than patients who developed non-RA persistent arthritis and those with resolving disease course. Similarly, lymphocyte apoptosis was absent in patients with early RA whereas it was seen in patients with other early arthritides. The mechanism for this inhibition of apoptosis may relate to the high levels of antiapoptotic cytokines (IL-2, IL-4, IL-15, granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor) found in the early rheumatoid joint [60].

Apoptosis proceeds through two major pathways: the intrinsic pathway is triggered by cellular stress, specifically mitochondrial stress caused by factors such as DNA damage and heat shock; and the extrinsic pathway is triggered by molecules released by other cells binding to transmembrane death receptors on the target cell to induce apoptosis (reviewed in [61]). A study in the K/BxN serum transfer model of inflammatory arthritis indicates that the proapoptotic protein Bid, an intermediary for the extrinsic and intrinsic apoptotic pathways, is important in the development of inflammatory arthritis. Mice lacking Bid display increased arthritis associated with more inflammation, pannus formation, bone destruction and infiltrating leukocytes. Furthermore there are fewer apoptotic cells in the joints of Bid^{-/-} compared with wildtype mice, suggesting that the failure to resolve arthritis in Bid^{-/-} mice may be due to an inability to delete autoreactive cells in the joint [62].

Most recently, the transcriptional activity in synoviocytes was investigated with a focus on the transcription factor Forkhead box class O isoforms, which are targets of the PI3 kinase/PKB signalling pathways and play emerging roles in the regulation of inflammatory responses. The Forkhead box class O isoforms are expressed in RA synovial tissue, and a strong negative correlation between inactivation (phosphorylation) of Forkhead box class O 4 in RA synovial tissue and increased serum C-reactive protein levels and raised erythrocyte sedimentation rate in RA patients has been demonstrated [63,64]. The Forkhead box class O isoforms may thus be involved in regulating homeostasis and inflammation in autoimmune diseases.

Regulatory T cells

Treg cells inhibit proliferation and cytokine production of conventional T cells, including self-reactive T cells, thereby controlling inflammatory responses and contributing to the maintenance of self-tolerance (reviewed in [65]). Although the frequency of CD4⁺CD25⁺ Treg cells is higher in the synovium fluid than in peripheral blood of RA patients, there is still persistent inflammation in the joint [66-70], suggesting that the Treg cells are ineffective in controlling inflammatory responses. There is increasing evidence that the suppressive function of these Treg cells is defective. CD4⁺CD25^{high} Treg cells isolated from patients with active RA express reduced levels of transcription factor forkhead box P3 (FoxP3) – which

plays a major role in the function of Treg cells [71,72] – that poorly suppress cytokine secretion from T cells and monocytes, and that do not convey a suppressive phenotype to effector CD4⁺CD25⁻ T cells [73,74]. A recent study by Valencia and colleagues suggests that TNF α , which is produced in RA synovium, inhibits the suppressive activity CD4⁺CD25⁺ Treg cells via signalling through TNF receptor II [73]. Interestingly, treatment with anti-TNF antibody (infliximab) increases FoxP3 expression in CD4⁺CD25^{high} Treg cells and restores their suppressive function [73,74]. Eliminating TNF α by antibody therapy might therefore be beneficial not only by directly suppressing proinflammatory processes but also by restoring the suppressive function of Treg cells.

Nadkarni and colleagues' work further suggests that anti-TNF therapy in RA patients generates a newly differentiated population of Treg cells, which compensates for the defective natural Treg cells [75]. The authors showed that infliximab treatment induced differentiation of a Treg cell population expressing FoxP3 and low levels of CD62L through conversion of CD4⁺CD25⁻ T cells. The natural CD62L⁺ Treg cells remained defective in infliximab-treated patients, whereas the infliximab-induced CD62L⁻ Treg cells mediated suppression via TGF β and IL-10 [75].

Using experimental animal models, it has been demonstrated that depletion of CD25⁺ T cells before or after the induction of arthritis leads to exacerbation of arthritis with increased cellular and humoral responses, and that transfer of CD4⁺CD25⁺ Treg cells at the time of induction of arthritis decreases the severity of disease. Transfer of Treg cells, however, appears unable to cure established chronic arthritis in animal models, suggesting that therapies increasing the number of Treg cells may not be sufficient to suppress ongoing inflammation in RA (reviewed in [76]). A novel immunoregulatory T-cell population was recently discovered in mice. Charbonnier and colleagues demonstrated that vaccination with immature dendritic cells suppresses CIA in mice and induces tolerance by expansion of an immunoregulatory TCR β ⁺CD49b⁺ T-cell population [77].

IL-6 and TGF β together induce differentiation of pathogenic Th17 cells from naïve T cells in mice [49]. TGF β is also a critical differentiation factor for generation of Treg cells [78], whereas IL-6, which is expressed in the RA synovium, was shown to inhibit TGF β -induced generation of FoxP3⁺ Treg cells [49]. The authors suggest not only that there is a functional antagonism between Th17 and Treg cells, but that these cells arise in a mutually exclusive fashion depending on whether they are activated in the presence of TGF β or TGF β plus IL-6.

B cells

B-cell depletion therapy with rituximab, an anti-CD20 monoclonal antibody, provides evidence that the proinflammatory

response in RA is dependent on the presence of B cells. CD20 is a B-cell surface antigen expressed only on pre-B cells and mature B cells that is lost before differentiation of B cells into plasma cells. A single course of two infusions of rituximab, alone or in combination with either cyclophosphamide or continued methotrexate, provided significant improvement in disease symptoms in RA patients [2]. Depletion of B cells may inhibit many different immunological responses as B cells are able to internalise, to process and to present antigens via MHC class II molecules to T cells, leading to T-cell activation and subsequent macrophage activation and further TNF α production.

In some RA patients, synovial B cells undergo differentiation and proliferation within extrafollicular germinal centres consisting of T-cell and B-cell aggregates [79]. A study using human synovium–SCID mouse chimeras showed that B cells are important to the formation of these germinal centres and follicular CD4⁺ T cells [4]. Moreover, activated B cells can produce proinflammatory cytokines and chemokines, and experiments in animal models of arthritis have demonstrated that activation of B cells via Toll-like receptors play a significant role in the development of arthritis (reviewed in [3]). Hence, there are many possible mechanisms and strategies of B-cell-directed therapies in autoimmune diseases. In the present review we shall focus on the role of B cells as plasma cells and on the increased interest in the role of autoantibodies in RA.

Autoantibodies

Several autoantibodies have been described in RA, but only rheumatoid factor (RF), antibodies to citrullinated antigens, and antibodies to immunoglobulin binding protein have shown sufficient sensitivity and specificity to be considered clinically useful (reviewed in [80]). RF is detectable in 70% to 80% of RA patients, but is also detectable in up to 10% of normal individuals and in other systemic diseases [80]. Antibodies to autoantigens modified by citrullination through deimination of arginine to citrulline are present in about two-thirds of all RA patients, but are rare (<2%) in healthy individuals and are relatively rare in other inflammatory conditions [81,82].

Although antibodies against citrullinated proteins are specific and predictive markers for rheumatoid arthritis, the pathologic relevance of these antibodies remains unclear. A recent study of the mouse CIA model demonstrated that antibodies against citrullinated proteins are involved in the pathogenesis of autoimmune arthritis. Kuhn and colleagues found that antibodies against both type II collagen and cyclic citrullinated peptide (CCP) appeared early after immunisation with type II collagen, before joint swelling was observed. When mice were tolerised with a citrulline-containing peptide prior to type II collagen challenge, a significantly reduced disease severity and incidence compared with control mice was demonstrated [83].

The controversy of whether autoantibodies contribute to, or are secondary to, the pathogenesis of RA was also recently addressed in a passive transfer model with mice deficient in the low-affinity inhibitory Fc receptor FcγRIIB. Petkova and colleagues showed that plasma or serum from patients with active RA induces inflammation and histological lesions in FcγRIIB^{-/-} mice consistent with arthritis, and it was caused by the IgG-rich fraction. In contrast, serum from normal blood donors did not induce arthritis. This suggests that humoral immunity can contribute directly to autoimmune arthritis [84]. Autoantibodies will be discussed further in the context of genetics, since studies suggest that the SE alleles of the HLA-DR gene are strongly associated with anti-CCP-positive RA but not with anti-CCP-negative RA.

Genetic risk factors

RA is a complex autoimmune disease that appears to be caused by small individual effects of many common genes rather than rare mutations of single genes, and a number of gene variations have been associated with autoimmunity and RA. The SE alleles of the HLA-DR gene comprise the major genetic risk factor for RA, whereas smoking is the major known environmental risk factor (reviewed in [85]). Other polymorphic genes thought to be involved in RA include protein tyrosine phosphatase N22 (PTPN22), CTLA4, peptidyl arginine deiminase type IV and macrophage migration inhibitory factor (reviewed in [86]). More recently, single-nucleotide polymorphisms (SNPs) within signal transducer and activator of transcription 4 (STAT4) [87,88] and TNF receptor-associated factor 1 (TRAF1)-C5 regions [89,90] were found to be associated with seropositive RA and other autoimmune diseases.

Protein tyrosine phosphatase N22

A polymorphism that results in a substitution of arginine with tryptophan (R620W) in the PTPN22 gene has been associated with RA in European and North American populations [91-93]. The amino acid substitution in PTPN22 (R620W) affects the gene's interaction with Src tyrosine kinases involved in regulation of T-cell receptor signalling in lymphocytes [94]. A recent study found that the PTPN22 variant R620W is associated with increased titres of IgG autoantibodies to an immunodominant conformational epitope (C1^{III}) of type II collagen in early RA [91]. The study also found that anti-C1^{III} titres were higher in RA patients harbouring alleles of the RA-associated HLA-DRB1 SE than in those lacking this SE. The allelic variants encoding the binding pocket for peptide presentation (SE) to T cells and a functional domain of a negative regulator of T-cell receptor signalling (PTPN22*620W), respectively, synergise in early RA to break the self-tolerance towards C1^{III}, an evolutionary conserved cartilage determinant.

The PTPN22 1858 SNP is also associated with future development of RA and has been shown to be a better predictor of RA than the HLA-SE [95]. Moreover, Johansson

and colleagues found that there was an association between PTPN22 1858 and anti-CCP antibodies and that the combination gives a specificity of 100% for diagnosing RA [95]. In a German cohort, the frequency of the PTPN22 1858 polymorphism was higher in male RA patients compared with female RA patients, indicating that this genetic contribution to pathogenesis might be more prominent in men [96]. Moreover, an association between PTPN22 and RA has been found in South Asians in the United Kingdom [97] but not in a Japanese population [98], suggesting that the PTPN22 gene is associated with RA only in specific genetic groups.

Signal transducer and activator of transcription 4

A genome-wide screen for RA-susceptible genes identified a region of 52 Mb genomic DNA on chromosome 2q that was associated with a risk of RA [99]. Fine mapping of this region in North American and Swedish populations recently revealed that four SNPs within the third intron of STAT4 were associated with risk of RA, rs7574865 being the most significant [88]. An association of rs7574865 and susceptibility of RA has subsequently also been found in a Korean population [87].

STAT4 is an intracellular molecule transducing signals triggered by IL-12, type I interferons and IL-23 (reviewed in [100]), and regulates the differentiation of Th1 and Th17 cells [101], the two lymphocyte subsets thought to be involved in the pathogenesis of many inflammatory diseases (reviewed in [102,103]). Perhaps it is not surprising that the same rs7574865 SNP was also associated with susceptibility of lupus (systemic lupus erythematosus), suggesting a common pathway of pathogenesis of autoimmune diseases [88].

TNF receptor-associated factor 1-C5

The SNPs in the region of the TRAF1-C5 locus on chromosome 9 have been associated with susceptibility and severity of RA in Dutch, Swedish and North American populations [89,90]. This genetic risk factor, however, was not identified in a genome-wide association study performed by the Wellcome Trust Case Control Consortium [104]. Association of SNPs within the TRAF1-C5 region with disease susceptibility and severity, however, is predominant in an autoantibody-positive subset of RA patients, suggesting this genetic risk factor is confined to a specific RA phenotype [89,90].

Shared epitope alleles

The SE alleles have been shown to increase cellular susceptibility to oxidative stress, which has been implicated in RA [105]. Ling and colleagues showed that the SE acts as an allele-specific ligand that activates nitric oxide-mediated pro-oxidative signalling in nearby cells, thereby increasing cell vulnerability to oxidative damage [106]. This activation may contribute to disease susceptibility and to severity of disease.

Recent studies suggest that SE alleles are strongly associated with anti-CCP-positive RA but not with anti-CCP-negative

RA, and are indeed more strongly associated with anti-CCP than with RA itself [107,108]. van der Helm-van Mil and colleagues further showed that the presence/absence of SE alleles correlates with the levels of anti-CCP antibodies, suggesting that the SE alleles act as classic immune response genes for the development of anti-CCP antibodies [107].

There are also data suggesting that anti-CCP and the RF status are independent severity factors for RA, with SE alleles playing a secondary role at most. RF and anti-CCP were strongly associated with radiographic severity of disease, and patients with both RF and anti-CCP expressed the most severe disease, suggesting both these factors may have important influence and pathways that lead to joint damage. The anti-CCP status was also strongly associated with the SE alleles and a clear gene dose–effect was observed. The magnitude of this effect was most striking in RF-negative patients, which supports the view that the association of SE with radiographic severity may be indirect and due to an association with anti-CCP [109].

There is increasing evidence that smoking is an environmental risk factor that, in the context of HLA-DR SE genes, may trigger RA-specific immune reactions to citrullinated proteins. A recent study by Klareskog and colleagues found that previous smoking is dose-dependently associated with occurrence of anticitrulline antibodies in RA patients and that the presence of SE genes was a risk factor only for anticitrulline-positive RA, and not for anticitrulline-negative RA. The combination of smoking history and the presence of double copies of HLA-DR SE genes increased the risk for RA 21-fold compared with the risk among nonsmokers carrying no SE genes. Moreover, positive immunostaining for citrullinated proteins was recorded in bronchoalveolar lavage cells from smokers but not in those from nonsmokers [108]. The gene–environment interaction between smoking and SE leading to autoantibodies has subsequently been reproduced in the Leiden case–control study [110] and in the Danish case–control study [111]. Unlike these studies, a recent study from North America could not, however, confirm an interaction between smoking, SE genes and anti-CCP, indicating that environmental factors other than smoking may also be associated with citrullination and RA [112].

Conclusions

Over the past couple of years advances have been made in the understanding of the involvement of T cells in the immunopathology of autoimmune diseases, with a focus on proinflammatory cytokine production, cell recruitment and osteoclastogenesis. *In vitro* investigations of Th17 cells have resulted in a better understanding of the T-cell inflammatory response and/or T-cell cytokine-driven inflammatory response in RA and their role in promoting osteoclastogenesis. *In vivo* studies with anti-TNF treatment indicated that Treg cells may be important in controlling inflammation. These investigations have identified new mechanisms of pathogenesis of RA and

have opened new possibilities for future therapeutic interventions. In addition, the genetic associations of the HLA-SE alleles with antibodies to citrullinated peptides in RA patients support a role of B cells in the pathogenesis of RA, although the precise mechanisms still remain unclear.

Competing interests

The authors declare that they have no competing interests.

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