The immunology of ankylosing spondylitis and rheumatoid arthritis: a tale of similarities and dissimilarities

R.D. Inman[1] and H.S. El-Gabalawy[2]

[1]University of Toronto, Ontario, Canada; [2]University of Manitoba, Winnipeg, Canada.

Robert D. Inman, MD, Professor of Medicine and Immunology
Hani S. El-Gabalawy, MD, Professor of Medicine and Immunology

Please address correspondence to:
Dr Robert D. Inman,
Professor of Medicine and Immunology,
1E-423, Toronto Western Hospital,
399 Bathurst St,
Toronto, Ontario
Canada M5T 2S8
E-mail: robert.inman@uhn.on.ca

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ABSTRACT

Ankylosing spondylitis (AS) and rheumatoid arthritis (RA) are immune-mediated inflammatory joint diseases with the potential for significant target organ damage. Genetic factors play an important role in defining disease susceptibility. Both diseases are mediated in part by TNF, since anti-TNF therapies have proved effective in both AS and RA. Despite their similarities, the genetic elements associated with the respective diseases differ, most notably in HLA associations, with AS being associated with class I HLA alleles and RA associated with class II HLA alleles. AS has a predilection for axial joints whereas RA targets peripheral joints, but the immunological basis of that distinction is unknown. Autoantibody formation is the immunological hallmark of RA, whereas AS is notable for being a "seronegative" disease. Growing knowledge of new aspects of the host immune response (such as innate immune responses and Th17 cells) is adding to new insights into shared mechanisms of pathogenesis between these two diseases.

Introduction

The early years of immunology were defined by resolving the effector arm of host defense against pathogens. Thus early clinical studies into the basis of chronic inflammation rheumatoid arthritis (RA) and ankylosing spondylitis (AS) were dominated by microbe hunting in the joints. Subsequently, with the recognition that these same effector pathways could turn pathogenic in autoimmune states, attention was focused on the recognition arm of immunity, in particular, self-nonself discrimination. The discovery of rheumatoid factors (RF) played an important role in this conceptual evolution. AS was originally called rheumatoid spondylitis, but the advent of RF testing bisected the field of inflammatory joint diseases into seropositive and seronegative domains, which has guided nomenclature to this day. Beyond the presence of RF, there are other important immunological differences between AS and RA, which will be the focus of this review.

Profiling immune cell populations

(i) T cells

T cells have long been the focus of intense investigation in RA, both in the systemic and the synovial compartments. Despite considerable heterogeneity in the results of the published studies, a number of broad conclusions have been reached regarding T cell populations in this disease. Most important has been the critical role for CD4 cells in RA synovitis. Multiple synovial biopsy studies have concluded the $CD4^+$ cells of the memory (CD45RO) phenotype are the predominant lymphocyte population in RA synovium, and that these are typically arranged in perivascular aggregates surrounding vessels with a high endothelial venule (HEV) morphology. In contrast, $CD8^+$ cells are located at the periphery of the lymphoid aggregates and immediately adjacent to the synovial lining cell layer. The CD4/CD8 ratio typically exceeds 4-5 in the synovial tissue compartment, and is highest in tissues featuring large lymphoid aggregates. In contrast, the CD4/CD8 ratio is lower in RA synovial fluid, with some studies reporting a predominance of $CD8^+$ cells. Detailed phenotypic and functional analyses of synovial $CD4^+$ T cells have suggested that although these cells are well equipped to provide effective B cell help, they have been found to be hyporesponsive to stimulation *in vitro* and to resemble anergic T cells (1). Moreover, the Vβ chain usage patterns have been shown to be relatively restricted suggesting oligoclonality, although they differ in different individuals. Past attempts to

categorize human synovial T cells by the Th1 (IFNγ)/Th2 (IL4) paradigm, which worked well in mouse models, have produced inconsistent and some unexpected results. Although it has been generally accepted that RA is a Th1 predominant disease, there is a relative absence of both IFNγ and IL4 in RA synovium. More recently, the identification of two other key T cell phenotypes, the pro-inflammatory Th17 T cells (2, 3) and the anti-inflammatory T regulatory cells (Treg) (4), has dramatically changed the paradigm in RA immunopathogenesis (5). There has been a rapid expansion of knowledge regarding the role of these two T cell subsets, and therapeutic strategies based on these pathways are in development.

One challenge facing students of AS is the relative inaccessibility of the target tissues. Biopsy of the sacroiliac joint, commonly the first site of inflammation, or the vertebral body, the focus of the chronic phase of disease, is not feasible in routine clinical settings. But a number of attempts have been undertaken. It should be pointed out that since a class I MHC association with AS is the dominant genetic susceptibility element, it was anticipated that $CD8^+$ CTL, restricted by HLA-B27, would be the predominant contributors to the local inflammation in the joints. This has largely not been borne out. The predominant T cells in joint infiltrates in the SI joints (6) and the zygoaphosyseal joints (7) or hip joints (8) have indicated abundant $CD4^+$ T cells as well as $CD8^+$ T cells. It is also notable that arthritis in the B27-transgenic rat model, which recapitulates several of the clinical features of spondyloarthritis, is transferrable not by $CD8^+$ T cells as anticipated, but by $CD4^+$ T cells (9). $CD8^+$ CTL, restricted by HLA-B27, were indeed found in reactive arthritis (ReA) with specificity for the inciting pathogen (10) but it proved difficult to apply this paradigm to AS. Recently it has been noted that $CD8^+$ T cells producing IL-4 are increased in AS, providing support for the notion that in B27+ individuals these cells are selectively expanded (11) The recognition that IL23R polymorphisms are associated with AS has created great interest in Th17 cells in this disease. It has recently been observed that in SpA peripheral blood there are increased numbers of circulating Th17 cells compared to RA, and that these cells are polyfunctional in terms of cytokine production (12).

(ii) B cells

In view of the key role that autoantibodies play in RA, it has long been assumed that B cells are central to the pathogenesis of this disease. This hypothesis has largely been confirmed by the dramatic therapeutic effects of B cell depletion in RA. Synovial tissues from patients with established RA typically demonstrate the presence of substantial numbers of B cells and plasma cells. The B cells are closely associated with $CD4^+$ T cells in the lymphoid aggregates, and a proportion of these aggregates exhibit typical germinal center morphology with the presence of follicular dendritic cells. Plasma cells on the other hand are located in areas peripheral to the lymphoid follicles in plasma cell rich zones. Although it remains unclear the extent to which B cell differentiation to plasma cells actually occurs within the synovial microenvironment, recent data suggest that synovial germinal centers are fully capable of maturing the development of autoantibodies such as ACPA (13). The relative proportion of autoantibody producing, long-lived plasma cells that are relatively resistant to the effects of B cell depletion also remains uncertain.

In the analysis of infiltrating cells in zygoapophyseal joints in AS it was noted that B cells made up a significant proportion of the cells (7). It may be that this cell population is playing a key role as antigen-presenting cells, rather than the precursors of humoral immunity, since plasma cells are notable for their absence and augmented immunoglobulin formation in general, and autoantibody formation in specific, are not hallmarks of AS.

(iii) Macrophages

The key role of macrophages in RA is now well established. TNF-α and IL-1β, the two cytokines at the hub of the RA cytokine network, are produced primarily by activated macrophages. Immunohistological analysis of RA synovium consistently reveals dramatic increases in the number of CD68+ macrophages, both in the lining cell layer and in the sublining stroma. It is assumed that this increase results primarily from recruitment of peripheral blood monocytes with subsequent differentiation and activation in the synovial compartment. Immunophenotyping studies have attempted for further define this process, but to date no conclusive evidence has emerged, particularly regarding the differential localization of M1 and M2 macrophages. It has though been proposed, based on the results of a number of synovial biopsy-based therapeutic trials, that the depletion of synovial macrophages in RA is a key biomarker associated with clinical response, irrespective of the mechanism of action of the therapeutic intervention (14, 15). This controversial hypothesis continues to be explored in the context of therapeutic trials using agents that have a novel mechanism of action and target new pathways.

While there have been relatively few studies on the immune events in axial inflammation, there has been an analysis of synovial immunopathology in peripheral joints which has shown a significant presence of CD163+ macrophages in the synovial infiltrates. The macrophage cytokine signature in SpA has different profile from RA, notably showing a selective decrease in M1-derived proinflammatory mediators such as TNF-α and IL-1β (16). Furthermore, studies on macrophage function in AS using microarray have revealed a profile of deficient IFN-γ production, and down-regulation of IFN-γ-regulated genes (17). Thus the macrophage presence in peripheral synovitis may have functions yet to be defined. One cautionary note on extrapolating the findings in peripheral joints to the spine is that these processes may be distinct. It has for example, been noted the peripheral synovitis in SpA is generally responsive to sulfasalazine therapy, but the axial inflammation is not (18). Microarray approaches to cytokine profiling have the advantage of not being restricted by pre-test hypotheses.

One such approach recently identified LIGHT as a potentially informative biomarker in AS (19).

Indirect clues to immunopathogenesis

(i) Autoantibody formation

In RA, the most compelling, albeit indirect, evidence for the autoimmune basis of the disease is the presence of autoantibodies, some of which are highly specific for RA. The two best studied autoantibody families are rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA), the former being directed towards the Fc portion of the IgG molecule, while the latter are directed towards citrulline containing peptide antigens. Both autoantibodies have been detected in pre-disease serum samples from RA patients, in some cases years before disease onset (20, 21). Indeed it now seems likely that most individuals who develop "seropositive" RA have one or both autoantibodies present at the time of disease presentation. In addition, as described above, it is now well documented that both autoantibodies are actively produced in RA synovium by follicular structures resembling the germinal centers found in lymphoid tissues.

A key question regarding these RA autoantibodies continues to be whether they are integrally involved in the pathogenesis of the disease, and specifically the pathogenesis of the chronic, destructive synovitis. Animal models such as the K/BxN serum transfer model have clearly indicated that autoantibodies, once formed can cause a persistent, destructive articular process as long as there is intact innate immunity (22, 23, 24). In contrast, it has been difficult to demonstrate the pathogenicity of the highly RA-specific ACPA is animal models. Recently it was shown that citrullinated human fibrinogen could precipitate a chronic destructive arthropathy in mice that were transgenic for HLA-DRB1*0401, the primary immunogenetic risk allele for RA in humans, although the role of ACPA was not directly demonstrated (25).

Another important and reproducible observation regarding the two predominant RA autoantibody systems, RF and ACPA, is that they are highly associated in RA patients, with the majority of individuals having established RA demonstrating both. Interestingly, it has been shown in a high risk cohort of the first degree relatives of Native American RA patients that although both RF and ACPA were prevalent and detectable in 15-20% of disease free individuals, there was minimal overlap between the two reactivities (26). These data suggest that RF and ACPA may arise through independent mechanisms that relate to specific gene-environment interactions, and that their development in a single individual may then increase the likelihood of precipitating synovitis, possibly through enhanced access of immune complexes into the joint. In turn, activation of innate immunity and inflammation would then expose intra-synovial citrullinated antigens such as cit-vimentin and cit-fibrnogen that would serve to locally evolve and amplify the ACPA responses. Of note, in the studies of the family members of Native American RA patients, anti-cit-vimetin (anti-Sa) and anti-cit-fibrinogen were detected exclusively in RA patients and not in the ACPA positive relatives.

Seronegative SpA has been operationally defined by the absence of rheumatoid factors in particular and autoantibodies in general. To support the relevance of this observation, it can be noted that hypcomplementemia is not a feature of active AS, and there is little evidence of local complement depletion in SF analyses in AS. It is also notable that citrullination of resident proteins in the inflamed synovium can be detected in SpA, but the necessary preconditions for a loss of tolerance to citrullinated proteins appear to be absent in this disease. AS patients have the capacity for antinuclear antibody formation, since this is a common occurrence following the institution of anti-TNF therapies, but the pathogenic significance of these autoantibodies is low, bona fide drug-induced lupus is rare. Nevertheless, this serological "adverse event", does indicate that the immune repertoire in AS is fully capable of autoantibody formation, given the appropriate stimulus. Pauci-immune from the autoimmunity perspective however should not be confused with a milder course. Chronic joint inflammation in the absence of RF can lead to dire clinical consequences, as evidenced by the bamboo spine of AS or the pencil-in-cup deformity of PsA.

(ii) MHC and disease susceptibility: clues to immunopathogenesis?

In the case of RA, the MHC association is with class II rather than class I molecules, and specific alleles of the HLA-DRB1 locus are associated with disease in essentially all populations studied. These disease associated alleles, which include HLA-DRB1*0401, *0404, *0405, *0408, *0101, *1001, and *1402, encode for a positively charged Q(K)RRAA motif in the third hypervariable region of the molecule located on the helical sidewall of the peptide binding groove, which has been popularly called the "shared epitope" (SE). The location and charge of this motif suggest that disease susceptibility relates to the capacity of these molecules to efficiently present specific peptide antigens to CD4 T cells, in particular citrullinated antigens. In support of this hypothesis, it has been shown that the SE containing MHC molecules *0401, *0404, *0101 have a substantially higher affinity for citrullinated compared to non-citrullinated (arginine containing) versions of peptide antigens derived from putative RA autoantigens such as vimentin (27). Moreover, in HLA-DRB1*0401 transgenic mice, robust T cell activation was seen when animals were stimulated with citrullinated compared to non-citrullinated vimentin peptides (27). These data, combined with a substantial body of human epidemiological research indicating that SE is associated only with ACPA positive RA, suggest that the SE encoding HLA-DRB1 alleles predispose to RA by facilitating a loss of tolerance to citrullinated endogenous antigens, and that this process occurs through the preferential presentation of these antigens to T cells. There is currently limited data in RA patients regarding the prevalence of T cell responses to citrullinated antigen, and whether these responses antedate the development of clinically detectable disease, as is the case with ACPA.

Although the SE/citrullinated antigen hypothesis is compelling, other competing hypotheses have been proposed to explain the association between HLA-DRB1 alleles and RA. The best substantiated of these hypotheses relates to the observation that if a negatively charged DERAA motif is present in the third hypervariable region of HLA-DRB1 rather than the positively charged SE motifs, there is actually protection from the development of RA, even in the presence of a SE allele. This has led to a "lack of protection" hypothesis, where the presence of an endogenous DERAA sequence is critical for maintaining tolerance to citrullinated antigens (27, 28). The HLA-DQ locus has also been proposed to play a role in the lack of protection hypothesis, in an analogous manner to HLA susceptibility to type I diabetes mellitus.

The strong association of HLA-B27 with AS has suggested several possible immune mechanisms which might link this susceptibility with the pathogenesis of disease. As mentioned above, presentation to arthritogenic T cells would be the canonical role assigned to a HLA class I-associated disease process. There has been progress made on defining the B27-binding motif and the related peptides which would be presented by B27 (29). But to date these peptide-binding profiles have not shown a discriminant association with AS-associated subtypes of B27 versus non-AS-associated subtypes (30). B27 itself might function as an autoantigen but it has been difficult to provide rigorous evidence of such a process using clinical materials. B27 may also mediate an immune-mediated disease process through other pathways. B27 has a propensity to misfold during assembly in the endoplasmic reticulum, and thereby can set the stage for an unfolded protein response (31). This may have important impact on cell physiology, in particular in resetting the threshold for upregulation of proinflammatory genes, such as TNF-α. Misfolding may contribute to disease pathogenesis in the B27 transgenic rat model of SpA (32) but alteration in this misfolding process did not reverse the joint inflammation in this model (33). Another distinctive feature of B27 is its tendency to form homodimers at the cell surface (34). These surface homodimers could function as peptide-presenting structures or could be recognized by particular receptors themselves. Finally, there is experimental support for the notion that B27 on the surface of antigen-presenting cells may serve to impair the immunological synapse between APC and responding T cell, perhaps through impairment of costimulatory molecule expression (35). None of these putative mechanisms is mutually exclusive and all could alter host immune responses. It is now evident that B27-positive status confers an enhanced immune defense against both HIV (36) and HCV (37), but how these lessons from infectious diseases might apply to the pathogenesis of AS is unclear.

(iii) Non-MHC genetic factors and immunity

In RA, candidate gene and genome wide association studies have identified a wealth of new genetic risk factors (38), most of which are involved in various aspects of immune regulation. To date, the best established of the non-MHC associations is with the PTPN22 gene, which encodes for a lymphocyte specific tyrosine phosphatase lyp that forms a regulatory complex with Csk kinase and serves to inhibit T cell receptor signaling (39,40). The missense SNP 1858C→T (620W) disrupts this interaction between lyp and Csk, thus preventing the formation of the lyp/Csk complex. It can be hypothesized that such a defect in a key T cell regulatory pathway may result in hyper-responsive T cells which in turn would predispose to a wide spectrum of autoimmune phenomenology. Indeed this SNP has been shown to be associated with multiple autoimmune disorders, including RA.

Although HLA-B27 is the dominant genetic influence in conferring susceptibility to AS, recent genetic studies have implicated other genes relevant to immunity. ERAP1 (endoplasmic reticulum associated aminopeptidase) has been shown to have associations with AS in American, Australian, British and Canadian populations (41, 42), as well as Korean AS patients (43). The effect is also seen in familial AS (44). This finding lends indirect support for the notion of presentation of arthritogenic peptides in the pathogenesis of AS since ERAP plays a key role in trimming of peptide in the ER so that they are appropriate for binding to class I MHC molecules. It is known that ERAP deficiency in experimental models alters host immune response to pathogens such as toxoplamosis, so effective host defense involves integrity of this peptidase and its peptide processing role (45). A second recent finding in genetics studies has been the association of IL23R polymorphisms with AS (41, 46). Unlike the ERAP studies, the IL23R polymorphisms were not associated with AS in Korea (47). This finding brings the Th17 pathway under scrutiny, as has been the case in psoriasis and inflammatory bowel disease. There has been a longstanding clinical impression of the role of gut immune events in the pathogenesis of AS, and recent findings have demonstrated upregulation of IL23 in gut mucosal tissues of AS patients without clinically overt IBD (48) so once again the link between the gut and the joint is under suspicion. And therapeutic intervention on this pathway has become a high priority, since mAb therapy directed against IL12/IL23R appears to be very promising.

(iv) Innate immunity

The first line of host defense against pathogens involves recognition of pathogen-associated molecular patterns, such as lipopolysaccharide. Amongst the receptors of the host innate immune response, toll-like receptors (TLR) have been the most extensively studied. Genetic variants in TLR4 have reported as associated with AS in some populations (49) but not in others (50). In reactive arthritis, which in some circumstances is the clinical precursor for AS, there has been an association with TLR2 polymorphism reported, suggesting that alteration in the first line of host defense may set the stage for later immune-mediated events (51). With respect to shedding light on mechanisms of disease, it has been observed using immunohistochemistry that there is an upregulation of TLR2 and TLR4 in the synovial

tissues of SpA patients in comparison with RA or OA controls (52). These same studies have also demonstrated decrease in local TLR expression concomitant with anti-TNF therapy, thus providing indirect support for an active role in the sustained inflammation in the joint in such patients (52).

The chronic synovitis seen in RA joints features a number of cells that express high levels of TLR, particularly dendritic cells, macrophages, and fibroblast-like synoviocytes (FLS) (53, 54). The relative contribution of each of these cell types to the initiation and perpetuation of RA synovitis continues to be investigated, both in human samples and animal models. Recent investigation has focused on TLR expression in FLS, resident cells that mediate the tissue response to inflammatory stimuli and are key to the destructive potential of RA synovitis. Compared to OA FLS, RA FLS express high levels of several TLR, in particular TLR2 and TLR4 (54, 55). *In vitro* ligation of these receptors induced the production of IL-6, chemokines, matrix metalloproteinases (MMP), and adhesion molecules (54-57). Together, this constellation of proinflammatory soluble mediators and cell surface molecules plays a central role in the persistence and destructiveness of RA synovitis.

The antigenic stimuli behind chronic inflammation

As summarized above, there is both direct and indirect evidence that immune-mediated processes play a central role in the chronic inflammation which is the hallmark of both AS and RA. But the nature of the antigenic stimulation either for triggering or sustaining these immune pathways over long periods of time has remained difficult to resolve. In the case of RA, the challenge of defining candidate exogenous and endogenous antigens has continued to attract investigators. An association between RF+ RA and immune responses to *P. mirabilis* and *E. coli* has been reported (58), although there is currently no clear mechanistic explanation for this. A hypothesis associating the periodontal pathogen *P gingivalis* with the development or amplification of ACPA (59), has recently been supported in some serological studies (60). It seems likely that multiple environmental stimuli, including smoking and microbial agents, can interact in genetically susceptible individuals to precipitate disease.

Because of the clinical interrelationships between ReA and AS, it has been proposed that AS may represent a chronic form of ReA, in which it may be difficult identifying the triggering pathogen if that event occurred in the remote past. *Klebsiella pneumoniae* is an example of a candidate arthritogenic pathogen, with supportive evidence for a role in AS deriving primarily from serological analysis of anti-Klebsiella antibodies. A systematic analysis of both humoral and cellular immune responses to *K. pneumoniae* in familial AS failed to demonstrate any specificity for an anti-Klebsiella signature which might reflect a primary role in AS for this pathogen (61). Epidemic ReA however does have the potential for chronicity, and there continues to be a sustained effort to identify persisting antigenic stimulation in such patients. Certainly there is no evidence that antibiotics constitute effective therapy for AS, at least rendering the persistence of viable microbes less likely. On the other hand, the antigenic stimulus in AS could come from endogenous rather than exogenous sources. It is recognized the mice immunized with either versican or aggrecan will develop a chronic arthritis which is notable for both axial and peripheral sites of inflammation (62). Furthermore, T cells responses, restricted by B27 and specific for the proteoglyan aggrecan, have been demonstrated in AS (63). Yet these cells are in relatively low frequency and it remains difficult to resolve the potential pathogenic potential of these cells. It may be that infection could play an indirect role, by modulating tolerance to self antigens. One approach to this question was a proof-of-principle study of autoreactive CTL directed against B27 in transgenic rats (64). In this study it was found that tolerance to self-B27 could be subverted by exposure to Chlamydia trachomatis, and that the autreactive epitope in B27 involved Lys70. Despite these experimental findings, it has proved difficult to establish a definitive role for autoreactive T cells in AS. Hence the nature of the inciting event for AS, as for RA, remains elusive.

References

1. ALI M, PONCHEL F, WILSON KE *et al.*: Rheumatoid arthritis synovial T cells regulate transcription of several genes associated with antigen-induced anergy. *J Clin Invest* 2001; 107: 519-28.
2. HARRINGTON LE, HATTON RD, MANGAN PR *et al.*: Interleukin 17-producing $CD4^+$ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005; 6: 1123-32.
3. PARK H, LI Z, YANG XO *et al.*: A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005; 6: 1133-41.
4. LEIPE J, SKAPENKO A, LIPSKY PE, SCHULZE-KOOPS H: Regulatory T cells in rheumatoid arthritis. *Arthritis Res Ther* 2005; 7: 93.
5. LUNDY SK, SARKAR S, TESMER LA, FOX DA: Cells of the synovium in rheumatoid arthritis. T lymphocytes. *Arthritis Res Ther* 2007; 9: 202.
6. BRAUN J, BOLLOW M, NEURE L *et al.*: Use of immunohistologic and in situ hybridization techniques in the examination of sacroiliac joint biopsy specimens from patients with ankylosing spondylitis. *Arthritis Rheum* 1995; 38: 499-505.
7. APPEL H, KUHNE M, SPIEKERMANN S *et al.*: Immunohistologic analysis of zygapophyseal joints in patients with ankylosing spondylitis. *Arthritis Rheum* 2006; 54: 2845-51.
8. APPEL H, KUHNE M, SPIEKERMANN S *et al.*: Immunohistochemical analysis of hip arthritis in ankylosing spondylitis: evaluation of the bone-cartilage interface and subchondral bone marrow. *Arthritis Rheum* 2006; 54: 1805-13.
9. BREBAN M, FERNÁNDEZ-SUEIRO JL, RICHARDSON JA *et al.*: T cells, but not thymic exposure to HLA-B27, are required for the inflammatory disease of HLA-B27 transgenic rats. *J Immunol* 1996 Jan 15; 156: 794-803.
10. HERMANN E, YU DT, MEYER ZUM BÜSCHENFELDE KH, FLEISCHER B: HLA-B27-restricted CD8 T cells derived from synovial fluids of patients with reactive arthritis and ankylosing spondylitis. *Lancet* 1993; 342: 646-50.
11. ZHANG L, JARVIS LB, BAEK HJ, GASTON JS: Regulatory $IL\text{-}4^+CD8^+$ T cells in AS patients and healthy controls. *Ann Rheum Dis* 2009; 68: 1345-51.
12. JANDUS C, BIOLEY G, RIVALS JP, DUDLER J, SPEISER D, ROMERO P: Increased numbers of circulating polyfunctional Th17 memory cells in patients with seronegative spondylarthritides. *Arthritis Rheum* 2008; 58: 2307-17.
13. HUMBY F, BOMBARDIERI M, MANZO A *et al.*: Ectopic lymphoid structures support ongoing production of class-switched autoantibodies in rheumatoid synovium. *PLoS Med* 2009; 6: e1.
14. GERLAG DM, HARINGMAN JJ, SMEETS TJ *et al.*: Effects of oral prednisolone on bio-

markers in synovial tissue and clinical improvement in rheumatoid arthritis. *Arthritis Rheum* 2004; 50: 3783-91.
15. HARINGMAN JJ, GERLAG DM, ZWINDERMAN AH *et al*.: Synovial tissue macrophages: a sensitive biomarker for response to treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 2005; 64: 834-8.
16. VANDOOREN B, NOORDENBOS T, AMBARUS C *et al*.: Absence of a classically activated macrophage cytokine signature in peripheral spondylarthritis, including psoriatic arthritis *Arthritis Rheum* 2009; 60: 966-75.
17. SMITH JA, BARNES MD, HONG D, DELAY ML, INMAN RD, COLBERT RA: Gene expression analysis of macrophages derived from ankylosing spondylitis patients reveals interferon-gamma dysregulation. *Arthritis Rheum* 2008; 58: 1640-9.
18. CLEGG DO, REDA DJ, ABDELLATIF M: Comparison of sulfasalazine and placebo for the treatment of axial and peripheral articular manifestations of the seronegative spondylarthropathies: a Department of Veterans Affairs cooperative study. *Arthritis Rheum* 1999; 42: 2325-9.
19. HAROON N, TSUI FW, O'SHEA FD *et al*.: From gene expression to serum proteins: biomarker discovery in ankylosing spondylitis.. *Ann Rheum Dis* 2008 Dec 22. Epub ahead of print.
20. NIELEN MM, VAN SD, REESINK HW *et al*.: Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004; 50: 380-6.
21. RANTAPAA-DAHLQVIST S, DE JONG BA, BERGLIN E *et al*.: Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003; 48: 2741-9.
22. JI H, OHMURA K, MAHMOOD U *et al*.: Arthritis critically dependent on innate immune system players. *Immunity* 2002; 16: 157-68.
23. MATSUMOTO I, STAUB A, BENOIST C, MATHIS D: Arthritis provoked by linked T and B cell recognition of a glycolytic enzyme. *Science* 1999; 286: 1732-5.
24. MONACH PA, VERSCHOOR A, JACOBS JP *et al*.: Circulating C3 is necessary and sufficient for induction of autoantibody-mediated arthritis in a mouse model. *Arthritis Rheum* 2007; 56: 2968-74.
25. HILL JA, BELL DA, BRINTNELL W *et al*.: Arthritis induced by posttranslationally modified (citrullinated) fibrinogen in DR4-IE transgenic mice. *J Exp Med* 2008; 205: 967-79.
26. IOAN-FACSINAY A, WILLEMZE A, ROBINSON DB *et al*.: Marked differences in fine specificity and isotype usage of the anti-citrullinated protein antibody in health and disease. *Arthritis Rheum* 2008; 58: 3000-8.
27. HILL JA, SOUTHWOOD S, SETTE A, JEVNIKAR AM, BELL DA, CAIRNS E: Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC class II molecule. *J Immunol* 2003; 171: 538-41.
28. FEITSMA AL, VAN DER HELM-VAN MIL AH, HUIZINGA TW, DE VRIES RR, TOES RE: Protection against rheumatoid arthritis by HLA: nature and nurture. *Ann Rheum Dis* 2008; 67 (Suppl. 3): iii61-iii63.
29. MERINO E, GALOCHA B, VÁZQUEZ MN, LÓPEZ DE CASTRO JA: Peptides: the cornerstone of HLA-B27 biology and pathogenetic role in spondyloarthritis. *Arthritis Rheum* 2008; 58: 3693-704.
30. MERINO E, GALOCHA B, VÁZQUEZ MN, LÓPEZ DE CASTRO JA: Disparate folding and stability of the ankylosing spondylitis-associated HLA-B*1403 and B*2705 proteins *Arthritis Rheum* 2008; 58: 3693-704.
31. COLBERT RA, DELAY ML, LAYH-SCHMITT G, SOWDERS DP: HLA-B27 misfolding and spondyloarthropathies. *Prion* 2009; 3: 15-26.
32. TURNER MJ, SOWDERS DP, DELAY ML *et al*.: HLA-B27 misfolding in transgenic rats is associated with activation of the unfolded protein response. *J Immunol* 2005; 175: 2438-48.
33. TRAN TM, DORRIS ML, SATUMTIRA N *et al*.: Additional human beta2-microglobulin curbs HLA-B27 misfolding and promotes arthritis and spondylitis without colitis in male HLA-B27-transgenic rats. *Arthritis Rheum* 2006; 54: 1317-27.
34. KOLLNBERGER S, BIRD LA, RODDIS M *et al*.: HLA-B27 heavy chain homodimers are expressed in HLA-B27 transgenic rodent models of spondyloarthritis and are ligands for paired Ig-like receptors. *J Immunol* 2004; 173: 1699-710.
35. HACQUARD-BOUDER C, CHIMENTI MS, GIQUEL B *et al*.: Alteration of antigen-independent immunologic synapse formation between dendritic cells from HLA-B27-transgenic rats and $CD4^+$ T cells: selective impairment of costimulatory molecule engagement by mature HLA-B27. *Arthritis Rheum* 2007; 56: 1478-89.
36. SCHNEIDEWIND A, BROCKMAN MA, SIDNEY J *et al*.: Structural and functional constraints limit options for cytotoxic T-lymphocyte escape in the immunodominant HLA-B27-restricted epitope in human immunodeficiency virus type 1 capsid. *J Virol* 2008; 82: 5594-605.
37. DAZERT E, NEUMANN-HAEFELIN C, BRESSANELLI S *et al*.: Loss of viral fitness and cross-recognition by $CD8^+$ T cells limit HCV escape from a protective HLA-B27-restricted human immune response. *J Clin Invest* 2009; 119: 376-86.
38. RAYCHAUDHURI S, REMMERS EF, LEE AT *et al*.: Common variants at CD40 and other loci confer risk of rheumatoid arthritis. *Nat Genet* 2008; 40: 1216-23.
39. VAN OM, WINTLE RF, LIU X *et al*.: Association of the lymphoid tyrosine phosphatase R620W variant with rheumatoid arthritis, but not Crohn's disease, in Canadian populations. *Arthritis Rheum* 2005; 52: 1993-8.
40. SIMINOVITCH KA: PTPN22 and autoimmune disease. *Nat Genet* 2004; 36: 1248-9.
41. WELLCOME TRUST CASE CONTROL CONSORTIUM; AUSTRALO-ANGLO-AMERICAN SPONDYLITIS CONSORTIUM (TASC): Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat Genet* 2007; 39: 1329-37.
42. MAKSYMOWYCH WP, INMAN RD, GLADMAN DD, REEVE JP, POPE A, RAHMAN P: Association of a specific ERAP1/ARTS1 haplotype with disease susceptibility in ankylosing spondylitis. *Arthritis Rheum* 29; 60: 1317-23.
43. CHOI CB, KIM TH, JUN JB *et al*.: ARTS1 polymorphisms are associated with ankylosing pondylitis in Koreans. *Ann Rheum Dis* 2009 May 3. Epub ahead of print.
44. TSUI FW, HAROON N, REVEILLE JD *et al*.: Association of an ERAP1 ERAP2 haplotype with familial ankylosing spondylitis. *Ann Rheum Dis* 2009 May 10. Epub ahead of print.
45. BLANCHARD N, GONZALEZ F, SCHAEFFER M *et al*.: Immunodominant, protective response to the parasite Toxoplasma gondii requires antigen processing in the endoplasmic reticulum. *Nat Immunol* 2008; 9: 937-44.
46. RAHMAN P, INMAN RD, GLADMAN DD, REEVE JP, PEDDLE L, MAKSYMOWYCH WP: Association of interleukin-23 receptor variants with ankylosing spondylitis. *Arthritis Rheum* 2008; 58: 1020-5.
47. SUNG IH, KIM TH, BANG SY *et al*.: IL-23R Polymorphisms in patients with ankylosing spondylitis in Korea. *J Rheumatol* 2009; 36: 1003-5.
48. CICCIA F, BOMBARDIERI M, PRINCIPATO A *et al*.: Overexpression of interleukin-23, but not interleukin-17, as an immunologic signature of subclinical intestinal inflammation in ankylosing spondylitis. *Arthritis Rheum* 2009; 60: 955-65.
49. SNELGROVE T, LIM S, GREENWOOD C *et al*.: Association of toll-like receptor 4 variants and ankylosing spondylitis: a case-control study. *J Rheumatol* 2007; 34: 368-70.
50. NA KS, KIM TH, RAHMAN P, PEDDLE L, CHOI CB, INMAN RD: Analysis of single nucleotide polymorphisms in Toll-like receptor 4 shows no association with ankylosing spondylitis in a Korean population. *Rheumatol Int* 2008; 7: 627-30.
51. TSUI FW, XI N, ROHEKAR S *et al*.: Toll-like receptor 2 variants are associated with acute reactive arthritis. *Arthritis Rheum* 2008; 58: 3436-8.
52. DE RYCKE L, VANDOOREN B, KRUITHOF E, DE KEYSER F, VEYS EM, BAETEN D: Tumor necrosis factor alpha blockade treatment down-modulates the increased systemic and local expression of Toll-like receptor 2 and Toll-like receptor 4 in spondylarthropathy. *Arthritis Rheum* 2005; 52: 2146-58.
53. HUANG Q, MA Y, ADEBAYO A, POPE RM: Increased macrophage activation mediated through toll-like receptors in rheumatoid arthritis. *Arthritis Rheum* 2007; 56: 2192-201.
54. KIM KW, CHO ML, LEE SH *et al*.: Human rheumatoid synovial fibroblasts promote osteoclastogenic activity by activating RANKL via TLR-2 and TLR-4 activation. *Immunol Lett* 2007; 110: 54-64.
55. BRENTANO F, SCHORR O, GAY RE, GAY S, KYBURZ D: RNA released from necrotic synovial fluid cells activates rheumatoid arthritis synovial fibroblasts via Toll-like receptor 3. *Arthritis Rheum* 2005; 52: 2656-65.
56. PIERER M, RETHAGE J, SEIBL R *et al*.: Chemokine secretion of rheumatoid arthritis

synovial fibroblasts stimulated by Toll-like receptor 2 ligands. *J Immunol* 2004; 172: 1256-65.

57. JUNG YO, CHO ML, KANG CM *et al*.: Toll-like receptor 2 and 4 combination engagement upregulate IL-15 synergistically in human rheumatoid synovial fibroblasts. *Immunol Lett* 2007; 109: 21-7.
58. NEWKIRK MM, GOLDBACH-MANSKY R, SENIOR BW *et al*.: Elevated levels of IgM and IgA antibodies to Proteus mirabilis and IgM antibodies to Escherichia coli are associated with early rheumatoid factor (RF)-positive rheumatoid arthritis. *Rheumatology* (Oxford) 2005; 44: 1433-41.
59. ROSENSTEIN ED, GREENWALD RA, KUSHNER LJ, WEISSMANN G: Hypothesis: the humoral immune response to oral bacteria provides a stimulus for the development of rheumatoid arthritis. *Inflammation* 2004; 28: 311-8.
60. MIKULS TR, PAYNE JB, REINHARDT RA *et al*.: Antibody responses to Porphyromonas gingivalis (P. gingivalis) in subjects with rheumatoid arthritis and periodontitis. *Int Immunopharmacol* 2009; 9: 38-42.
61. STONE MA, PAYNE U, SCHENTAG C, RAHMAN P, PACHECO-TENA C, INMAN RD: Comparative immune responses to candidate arthritogenic bacteria do not confirm a dominant role for Klebsiella pneumonia in the pathogenesis of familial ankylosing spondylitis. *Rheumatology* (Oxford). 2004; 43: 148-55.
62. GLANT TT, MIKECZ K: Proteoglycan aggrecan-induced arthritis: a murine autoimmune model of rheumatoid arthritis. *Methods Mol Med* 2004; 102: 313-38.
63. KUON W, KUHNE M, BUSCH DH *et al*.: Identification of novel human aggrecan T cell epitopes in HLA-B27 transgenic mice associated with spondyloarthropathy. *J Immunol* 2004; 173: 4859-66.
64. POPOV I, DELA CRUZ CS, BARBER BH, CHIU B, INMAN RD: Breakdown of CTL tolerance to self HLA-B*2705 induced by exposure to Chlamydia trachomatis. *J Immunol* 2002; 169: 4033-8.