Review

Roles of B cells in rheumatoid arthritis

Gregg J Silverman and Dennis A Carson

Rheumatic Disease Core Center and Division of Rheumatology, Allergy and Immunology, Department of Medicine, University of California San Diego, La Jolla, California, USA

Correspondence: Gregg J Silverman (e-mail: gsilverman@ucsd.edu)

Received: 9 Sep 2003 Accepted: 6 Oct 2003 Published: 2 Dec 2003

Arthritis Res Ther 2003, **5(Suppl 4)**:S1-S6 (DOI 10.1186/ar1010)

Abstract

B lymphocytes play several critical roles in the pathogenesis of rheumatoid arthritis. They are the source of the rheumatoid factors and anticitrullinated protein antibodies, which contribute to immune complex formation and complement activation in the joints. B cells are also very efficient antigen-presenting cells, and can contribute to T cell activation through expression of costimulatory molecules. B cells both respond to and produce the chemokines and cytokines that promote leukocyte infiltration into the joints, formation of ectopic lymphoid structures, angiogenesis, and synovial hyperplasia. The success of B cell depletion therapy in rheumatoid arthritis may depend on disruption of all these diverse functions.

Keywords: antigen presentation, autoantibody, immune complexes, immunity, synovitis, tolerance

Introduction

IgG autoantibodies and immune complexes have long been recognized as potent pathologic triggers of inflammatory responses. Early hypotheses regarding the pathogenesis of rheumatoid arthritis (RA) were molded by experimental models of immune complex disease. IgG aggregates are abundant in RA synovial fluids, and can trigger complement activation. Hence, it seemed logical that tissue damage in RA was attributable to the local deposition of immune complexes.

Rheumatoid factors (RFs), which are autoantibodies specific for the constant regions of IgG, are detectable in more than 80% of RA patients, and may also be present in a 'hidden' or complexed form in the synovial fluids of some seronegative patients. RFs efficiently fix and activate complement *in vitro* by the classic pathway [1]. *In vivo* turnover studies of radiotagged complement proteins in seropositive RA patients demonstrated that, compared with control individuals, complement consumption was greatly accelerated, especially at the extravascular sites of inflammation [2]. Consistent with the notion that immune complex formation was maximal at the synovial sites of inflammation, complement activation was shown to be much greater in RA synovial fluid than in blood [2–4]. Levels of C4 fragments at these sites also correlated with titers of IgM RFs.

Infiltrating leukocytes are known to be recruited by the downstream products of complement activation, especially the soluble anaphylatoxin C5a, with subsequent enlistment of other components of the membrane attack complex [5,6]. Flares of clinical activity in RA correlate with increased levels of RF secreting cells, which are especially prevalent in the bone marrow and synovial fluid of RA patients [7].

IgM RFs have been reported to account for more than 10% of local plasma cells in RA synovia [8,9]. However, infusions of RF into healthy individuals causes neither sustained nor transient synovitis [10], indicating that RF autoantibodies by themselves are not pathogenic. Nevertheless, IgM-RF-containing immune complexes may also include IgG antibodies and unidentified peptides, which could derive from self or exogenous antigens. In addition, the recently resolved crystallographic structure of a human

APC = antigen-presenting cell; CCP = citrulline-modified peptides and proteins; CXCL = CXC ligand; GC = germinal center; GPI = glucose-6-phosphate isomerase; IFN = interferon; IL = interleukin; RA = rheumatoid arthritis; RF = rheumatoid factor; SDF = stromal cell derived factor; TNF = tumor necrosis factor.

IgM RF–Fc co-complex revealed that contacts with IgG antigen involved only the periphery of the antigen-binding cleft of the autoantibody [11]. These findings may indicate that RA RF is capable of binding IgG as well as another self or foreign antigen. Thus, although RF alone is not proinflammatory, RF associated with immune complexes can enhance local inflammatory processes, and there is compelling clinical evidence that RFs contribute to extra-articular disease.

Antigen-presenting function of B cells

In addition to being the precursors of antibody-secreting plasma cells, the B cells in RA can play a critical role in the afferent arm of the immune response (Table 1). Thus, B cells can act as highly efficient antigen-presenting cells (APCs), supporting the activation of autoreactive T cells. In fact, by virtue of the high affinity of a specific membrane-associated immunoglobulin for antigen, an antigen-specific B cell can take up, process, and present peptides from nominal antigen with 1000-fold or greater efficiency than a 'professional' APC. In addition, activated B cells can synthesize cytokines and membrane-associated molecules that provide nonspecific help to adjacent T cells.

Strong experimental support for a central role of B cells in RA pathogenesis, independent of antibody formation, came from studies of human synovium/SCID mouse chimeras [12]. Those studies confirmed that the T cell activation is B cell dependent, in that targeted deletion of B cells impaired local T cell responsiveness, and APCs other than B cells could not substitute for the maintenance of T cell activation.

Role of B cells in murine models of rheumatoid arthritis

Following the development of methods for introduction of transgenes and for targeted gene disruption, *in vivo* murine models have been developed that have enabled a re-evaluation of the role of B cells in arthritis. The KRN/NOD murine model has been particularly revealing, because in these mice there is complete penetrance of a genetically determined disease process that results in severe distal joint inflammation, which emulates key features of RA [13]. Moreover, development of the disease involves the coordinated functions of both B cells and T cells, because infusion of nondepleting antibody to CD4 blocks disease, and mice devoid of B cells also do not develop disease [13]. A pathogenic role for IgG autoantibodies to glucose-6-phosphate isomerase (GPI) has been documented in the KRN/NOD model; it was found that infusions of these autoantibodies into otherwise healthy mice rapidly led to the development of synovitis [14]. However, arthritis was not induced by introduction of preformed anti-GPI antibody–antigen complexes, which suggested that joint disease can result when autoantibodies, produced either locally or systemically, interact with antigens on synovial surfaces to develop immune complexes *in situ*. These locally formed immune complexes were shown to be proinflammatory only if they involve antibodies to two or more epitopes, and the more diverse the antibody response, the more efficient the recruitment of proinflammatory factors [15], suggesting that a complex IgG autoantigen lattice is required for the recruitment of downstream inflammatory effectors.

Table 1

Major physiologic functions of B lymphocytes

Precursors of antibody producing plasma cells
Provide noncognate help for T cell activation
Efficient antigen-presenting cells, especially for recall antigens
Produce cytokines (i.e. IL-4 and IL-10) that support the survival other mononuclear cells
Generate and respond to chemotactic factors responsible for leukocyte migration and development of granulation tissue
Sustain immunologic memory

Studies in the KRN/NOD model have also enabled a closer dissection of the role of complement in the development of immune complex mediated synovitis. Although anti-GPI antibodies are prevalent in the circulation, disease is limited to the joints, probably because only articular immune complexes are efficient at fixing complement [14]. C4-deficient KRN/NOD mice develop arthritis of the same severity as do wild-type mice, whereas animals deficient in factor B of the alternative complement pathway develop attenuated disease or no arthritis at all [16,17]. In addition, a partial dependence on C3 was also demonstrated [16], which is consistent with the known role of C3 in the stabilization of immune complexes. These findings have led to a revision of the earlier notion that IgG immune complexes trigger inflammation primarily by engagement of the classical pathway of complement activation, because these murine studies indicated that disease initiation instead may involve the alternative pathway.

Although interactions between immune complexes and cellular receptors for the Fc regions of IgG (FcγR) were not considered in earlier clinical investigations of RA, the recent characterization of these receptors and the availability of relevant murine model systems has permitted a thorough examination of their impact on pathogenesis. The three classes of Fcγ cell surface receptors, isolated based on their capacity to bind IgG-containing immune complex, are heterogeneous in their binding specificities for IgG_1–IgG_4, and their intracellular signaling motifs that can either activate or inhibit cellular effector functions. Although mice with deficiencies in complement components typically exhibit attenuated immune complex

mediated disease in relevant murine models, the loss of activating FcγRIII completely ablates arthritis development [18]. Connecting these model systems to clinical disease, *in vitro* blockade of FcγRIIIA on human macrophages was recently shown to prevent the release of tumor necrosis factor (TNF)-α and induction of IL-1α in human macrophages [19]. FcγRIII may be an especially important mediator of immune complex induced tissue damage in RA, because of the size and composition of immune complexes that arise at sites of disease (discussed by Edwards and coworkers [20]). In more recent studies, C5a, acting through the C5a receptor, was shown to exacerbate immune complex induced inflammatory disease by altering the ratio of activating to inhibitory FcγR on macrophages [21]. These studies demonstrated a direct link between the C5a chemoattractant and FcγR related mechanisms responsible for immune complex triggered inflammatory responses.

These investigations of murine models of RA have led to a revision of our understanding of the role of secreted autoantibodies in RA. Complement alone may be inadequate to sustain chronic inflammatory responses to immune complex deposition, because stimulatory and inhibitory FcγR may in fact be the primary regulators of subsequent immune responses (for review [21]). The host response to immune complex functions in the joints is critically dependent on interactions with cell surface FcγR on B cells and macrophages.

Immune complexes and mast cells

Mast cells are prominent in the synovial infiltrates of many RA patients [22], and these tissue associated infiltrating cells express membrane associated FcR for IgE and IgG, which enable triggering resulting from antigen-specific (and perhaps nonspecific) immunoglobulin cross-linking. In murine models of immune complex disease, the mast cell has been shown to play a central role in immune complex mediated joint inflammation [23]. Mast cells triggered by immune complexes produce proinflammatory cytokines and proteolytic enzymes at sites of cartilage erosions [24]. The mast cells are also major sources of the vasoactive and chemotactic factors that facilitate the recruitment of other leukocytes to the synovial tissues. KRN/NOD mice that lack mast cells are resistant to inflammatory and erosive arthritis induced by arthritogenic serum [25]. Along with macrophages, mast cells can release TNF-α and IL-1. For these reasons, the mast cell is now appreciated to be a cellular link between B cells, autoantibodies, complement, and other inflammatory mediators that contribute to erosive arthritis.

Anti-GPI antibodies and rheumatoid arthritis

Based on the unexpected discovery that antibodies to GPI can induce synovitis in healthy mice, several groups have looked for a role for these autoantibodies in clinical disease. GPI is a ubiquitous enzyme that is essential for glucose metabolism in all cell types, and it therefore cannot represent a tissue-specific antigen recognized as part of an autoimmune disease that is limited to the joints. A more likely scenario is that increased local cell turnover results in the deposition of GPI along joint surfaces [14], where it may be recognized by autoantibodies that enter from the circulation. Although Schaller and coworkers [26] reported increased anti-GPI antibody titers in 64% of 69 RA patients, but not in patients with Lyme disease or Sjögren's syndrome, these findings were not confirmed in subsequent reports. In a recent flurry of studies, only a small minority of RA patients had detectable levels of autoantibodies to GPI, and there were no significant differences from patients with other types of joint diseases [27–30]. However, it remains a possibility that anti-GPI antibodies may be more common in RA patients with extra-articular disease, and especially in patients with Felty's syndrome, which were highly represented in the first reported clinical survey [26].

Antibodies against citrulline-modified proteins

Many RA patients have circulating antibodies to autoantigens other than IgG, including type II collagen, heat shock proteins, proteoglycans, cartilage link protein, and heavy chain binding proteins [31]. In some cases T cell reactivity to the same antigens was demonstrated by *in vitro* proliferation assays [32]. In certain instances experimental immunization with the antigens in complete Freund's adjuvant can cause arthritis in rodent models [33]. However, immune responses to each of these antigens have been detected in only a minority of patients, and a causative role in clinical disease has not been firmly established. Therefore, at best, such joint-specific autoantibodies cannot account for chronic inflammatory arthritis in most RA patients.

More prominent in RA are IgG antibodies to citrulline-modified peptides and proteins (CCP). Citrullination represents a post-translational modification due to the enzymatic deamination of peptidyl arginine to peptidyl citrulline. It has been shown that citrulline is the essential antigen epitope recognized by anti-CCP antibodies, antiperinuclear antibodies, as well as antibodies to keratin, filaggrin, and Sa. In fact, one extensive study [34] concluded that although RF may be a more sensitive test for RA, detection of anti-CCP antibodies is more than 90% specific for the diagnosis of RA. Hence, the available evidence supports a role for serologic testing for anti-CCP antibodies as an aid in the diagnosis of RA, especially at early stages of disease [35,36].

Although the biologic significance of anti-CCP antibodies is unclear, citrullination may be a byproduct of abnormal protein metabolism occurring at *in vivo* sites of increased or abnormal apoptosis [37]. The B lymphocytes from RA

patients are resistant to certain apoptotic stimuli [38], which may reflect prosurvival signals delivered from synovial or bone marrow stromal cells [39]. Notably, increased production of citrullinated peptide autoantibodies has been demonstrated in two murine model systems of autoimmunity with abnormalities in B cell apoptosis, whereas anti-CCP responses were not associated with other autoimmune models that included the classical collagen-induced arthritis system [40]. These findings may suggest that the generation of autoantibodies to citrulline related neoantigens could be closely linked to mechanisms responsible for impaired lymphocyte clonal regulation [34].

Citrullination of proteins may also have the potential to contribute directly to the autoimmune response [41]. An unexpected mechanistic linkage was found in studies of mice transgenic for the human HLA-DRB1*0401 MHC class II molecule, which contains the 'shared epitope' – a well studied genetic susceptibility factor for RA. *In vitro* incubation of transgenic B cells and macrophages with citrulline-containing peptides resulted in enhanced peptide side chain interactions with the shared epitope that significantly increased peptide MHC affinity. Moreover, immunization of the HLA-DRB1*0401 transgenic mice led to greatly enhanced *in vitro* recall responses to synthetic citrullinated self peptides [41]. These observations suggest a new mechanism by which the 'shared epitope' can predispose to RA.

Ectopic lymphoid tissue in rheumatoid arthritis

About 60% or more of the synovial samples from RA patients have infiltrates of B and T lymphocytes. Three separate patterns of infiltrates have been described: diffuse lymphocytic infiltrates with interdigitating dendritic cells and variable amounts of B cells; aggregates of infiltrating B and T cells in more substantial numbers, associated with interdigitating dendritic cells in disorganized groupings; and T cells and B lymphocytes clustered in aggregates arrayed around interdigitating dendritic cells and associated with follicular dendritic cell networks. In this latter pattern, which is present in less than one-third of patients, the synovial cellular infiltrates appear to be organized into distinct B cell follicle-like structures in close spatial relationship to $CD4^+$ T cells and $CD8^+$ T cells. In general, these histologic features are similar to the germinal center (GC) reactions that arise in peripheral lymphoid tissues during antigen-specific responses after immunization [42]. Immunohistochemical analyses have also shown that within the lymphocytic infiltrates in the synovial tissue, plasma cells may be organized in concentric rings around large cellular clusters of T cells and $CD20^+$ B cells, or as perivascular clusters [43]. In addition, B cells and T cells in rheumatoid synovial samples may bear the proliferation associated cell marker Ki-67. This antigen has been detected primarily on lymphocytes in the GC-like pattern [44], but concurrent mitotic figures in RA synovial intima are very low, suggesting that local B or T cell proliferative expansions may be quite limited and/or that expression of this marker is not synonymous with proliferation. Moreover, B cell proliferation in ectopic lymphoid infiltrates has been seen mainly in the network of the follicular dendritic cell, whereas in human tonsilar GC reactions proliferation occurs in a separate dark zone [45]. These findings suggest that ectopic GC-like structures have functional and organizational differences from their physiologic analogs.

Although the presence of lymphoid infiltrates generally correlates with clinical disease in RA, there is currently limited information regarding the natural history of these histologic patterns [46], and it is unknown whether a particular histologic pattern has implications for prognosis or treatment responsiveness. Furthermore, although the diseased joints in RA patients may have organized lymphoid infiltrates that emulate physiologic peripheral lymphoid tissues, it is still not clear that these changes are entirely specific for RA. The affected joints of patients with ankylosing spondylitis have also been shown at times to harbor GC-like aggregates [47]. Even the synovia from osteoarthritic joints can occasionally contain infiltrates of activated B cells and plasma cells exhibiting clonally related antibody gene sequences [48,49]. In addition, lymphotoxin-β (LT-1β2), which is produced by activated T cells, may be a downstream effector that is required for the development of primary B cell follicles in the synovial infiltrates [50–52]. Rheumatoid synovial tissues have also unexpectedly been found to be rich sources of CXCL12 (also termed stromal cell derived factor [SDF]-1) [53]. In fact, SDF-1 produced by fibroblast-like synoviocytes has been shown to contribute to the resistance of B cells to apoptosis, which supports an earlier hypothesis that specialized synovial 'nurse-like cells' peculiar to RA synovium mediate homing and survival of B cells [54]. In recent studies plasma cells have also been shown to migrate toward gradients of SDF-1 [55], CXCL9 (monokine induced by IFN-γ), CXCL10 (IFN-γ-inducible protein 10), and CXCL11 (IFN-inducible T cell α chemoattractant) [55]. SDF-1, as well as IL-5, IL-6, TNF-α, and ligands for CD44, can also prolong the longevity of plasma cells [56]. In addition, the TNF-α family member B lymphocyte stimulator (BLyS, also called Baff) has also recently been detected at high levels in rheumatoid synovial fluid, suggesting that this prosurvival factor can also be locally produced in inflamed joints [57].

The dysregulated expression of cytokines and costimulatory molecules explains the reported accumulation in one-third of RA samples of an unusual subpopulation of peripheral B cells with a restricted immunoglobulin variable region gene repertoire that coexpress conventional light chains and the surrogate light chain of pre-B cells [58]. Such B lineage cells have been postulated to be

Table 2

Potential pathologic functions of B lymphocytes in autoimmune disease

Presentation of immune-complexed antigens to autoreactive T cells
Expression of adhesion and other costimulatory molecules that promote T cell activation
Synthesis of chemokines that induce leukocyte infiltration
Production of factors that initiate and sustain angiogenesis and granulation tissue formation
Release of autoantibodies that are directly or indirectly (via immune complex formation) destructive to tissues
Maintenance of a memory response to autoantigens

promiscuous in antigen presentation, and thus could present diverse autoantigens. This topic is still highly controversial because other investigators have not found these B lineage cells in healthy tissues, and have challenged the authenticity of their reported phenotype [59].

The imprint of the potent chemoattractive activating and antiapoptotic factors described above may largely explain the skewed distribution of the B lymphoid cells that accumulate in the synovium. The local production of these factors at sites of inflammation in RA joints may serve as beacons to foster B cell accumulation, proliferation, and differentiation. An active (auto)antigen driven GC reaction may not be required to explain the development of the ectopic B lymphoid infiltrates in the joints of RA patients.

Conclusion

Recent reports have confirmed the importance of immune complexes in the pathogenesis of RA, and have elucidated additional critical roles for B cells and their immunoglobulin products in self-sustaining chronic inflammatory processes (Table 2). These findings have contributed to the rationale for the development of targeted therapies that delete B cells [60] or that attenuate the function of secreted and membrane associated factors that contribute to B cell accumulation and survival at sites of disease [61]. However, an appreciation of these diverse potential roles may also predict that the targeted interference with just one B cell antigen or costimulatory molecule may not be sufficient to arrest ongoing disease in all RA patients.

Competing interests

None declared.

Acknowledgements

The work described here is supported in part by grants AI40305, AR47360, and AR44850 from the National Institutes of Health, and a grant from the Alliance for Lupus Research.

References

1. Vaughan JH, Chihara T: **Lymphocyte function in rheumatic disorders.** *Arch Intern Med* 1975, **135:**1324-1328.
2. Kaplan RA, Curd JG, Deheer DH, Carson DA, Pangburn MK, Muller-Eberhard HJ, Vaughan JH: **Metabolism of C4 and factor B in rheumatoid arthritis. Relation to rheumatoid factor.** *Arthritis Rheum* 1980, **23:**911-920.
3. Ruddy S, Britton MC, Schur PH, Austen KF: **Complement components in synovial fluid: activation and fixation in seropositive rheumatoid arthritis.** *Ann NY Acad Sci* 1969, **168:**161-172.
4. Ruddy S, Austen KF: **The complement system in rheumatoid synovitis. I. An analysis of complement component activities in rheumatoid synovial fluids.** *Arthritis Rheum* 1970, **13:**713-723.
5. Goldstein I M, Weissmann G: **Generation of C5-derived lysosomal enzyme-releasing activity (C5a) by lysates of leukocyte lysosomes.** *J Immunol* 1974, **113:**1583-1588.
6. Borel JF, Keller HU, Sorkin E: **Studies on chemotaxis. XI. Effect on neutrophils of lysosomal and other subcellular fractions from leukocytes.** *Int Arch Allergy Appl Immunol* 1969, **35:**194-205.
7. Tanimoto K, Cooper NR, Johnson JS, Vaughan JH: **Complement fixation by rheumatoid factor.** *J Clin Invest* 1975, **55:**437-445.
8. Youinou PY, Morrow JW, Lettin AW, Lydyard PM, Roitt IM: **Specificity of plasma cells in the rheumatoid synovium. I. Immunoglobulin class of antiglobulin-producing cells.** *Scand J Immunol* 1984, **20:**307-315.
9. Fehr K, Velvart M, Rauber M, Knopfel M, Baici A, Salgam P, Boni A: **Production of agglutinators and rheumatoid factors in plasma cells of rheumatoid and nonrheumatoid synovial tissues.** *Arthritis Rheum* 1981, **24:**510-519.
10. Harris J, Vaughan JH: **Transfusion studies in rheumatoid arthritis patients.** *Arthritis Rheum* 1961, **4:**47-55.
11. Sutton BJ, Corper AL, Sohi MK, Jefferis R, Beale D, Taussig MJ: **The structure of a human rheumatoid factor bound to IgG Fc.** *Adv Exp Med Biol* 1998, **435:**41-50.
12. Takemura S, Klimiuk PA, Braun A, Goronzy JJ, Weyand CM: **T cell activation in rheumatoid synovium is B cell dependent.** *J Immunol* 2001, **167:**4710-4718.
13. Kouskoff V, Korganow AS, Duchatelle V, Degott C, Benoist C, Mathis D: **Organ-specific disease provoked by systemic autoimmunity.** *Cell* 1996, **87:**811-822.
14. Matsumoto I, Maccioni M, Lee DM, Maurice M, Simmons B, Brenner M, Mathis D, Benoist C: **How antibodies to a ubiquitous cytoplasmic enzyme may provoke joint-specific autoimmune disease.** *Nat Immunol* 2002, **3:**360-365.
15. Maccioni M, Zeder-Lutz G, Huang H, Ebel C, Gerber P, Hergueux J, Marchal P, Duchatelle V, Degott C, van Regenmortel M, Benoist C, Mathis D: **Arthritogenic monoclonal antibodies from K/BxN mice.** *J Exp Med* 2002, **195:**1071-1077.
16. Ji H, Ohmura K, Mahmood U, Lee DM, Hofhuis FM, Boackle SA, Takahashi K, Holers VM, Walport M, Gerard C, Ezekowitz A, Carroll MC, Brenner M, Weissleder R, Verbeek JS, Duchatelle V, Degott C, Benoist C, Mathis D: **Arthritis critically dependent on innate immune system players.** *Immunity* 2002, **16:**157-168.
17. Solomon S, Kolb C, Mohanty S, Jeisy-Walder E, Preyer R, Schollhorn V, Illges H: **Transmission of antibody-induced arthritis is independent of complement component 4 (C4) and the complement receptors 1 and 2 (CD21/35).** *Eur J Immunol* 2002, **32:**644-651.
18. Sylvestre D, Clynes R, Ma M, Warren H, Carroll MC, Ravetch JV: **Immunoglobulin G-mediated inflammatory responses develop normally in complement-deficient mice.** *J Exp Med* 1996, **184:**2385-2392.
19. Abrahams VM, Cambridge G, Lydyard PM, Edwards JC: **Induction of tumor necrosis factor alpha production by adhered human monocytes: a key role for Fcgamma receptor type IIIa in rheumatoid arthritis.** *Arthritis Rheum* 2000, **43:**608-616.
20. Edwards JC, Cambridge G, Abrahams VM: **Do self-perpetuating B lymphocytes drive human autoimmune disease?** *Immunology* 1999, **97:**188-196.
21. Shushakova N, Skokowa J, Schulman J, Baumann U, Zwirner J, Schmidt RE, Gessner JE: **C5a anaphylatoxin is a major regulator of activating versus inhibitory FcgammaRs in immune complex-induced lung disease.** *J Clin Invest* 2002, **110:**1823-1830.
22. Crisp AJ, Chapman CM, Kirkham SE, Schiller AL, Krane SM: **Articular mastocytosis in rheumatoid arthritis.** *Arthritis Rheum* 1984, **27:**845-851.
23. Sylvestre DL, Ravetch JV: **A dominant role for mast cell Fc receptors in the Arthus reaction.** *Immunity* 1996, **5:**387-390.
24. Tetlow LC, Woolley DE: **Mast cells, cytokines, and metalloproteinases at the rheumatoid lesion: dual immunolocalisation**

studies. *Ann Rheum Dis* 1995, **54:**896-903.
25. Lee DM, Friend DS, Gurish MF, Benoist C, Mathis D, Brenner MB: **Mast cells: a cellular link between autoantibodies and inflammatory arthritis.** *Science* 2002, **297:**1689-1692.
26. Schaller M, Burton DR, Ditzel HJ: **Autoantibodies to GPI in rheumatoid arthritis: linkage between an animal model and human disease.** *Nat Immunol* 2001, **2:**746-753.
27. Kassahn D, Kolb C, Solomon S, Bochtler P, Illges H: **Few human autoimmune sera detect GPI.** *Nat Immunol* 2002, **3:**411-412; author reply 412-413.
28. Schubert D, Schmidt M, Zaiss D, Jungblut PR, Kamradt T: **Autoantibodies to GPI and creatine kinase in RA.** *Nat Immunol* 2002, **3:**411; author reply 412-413.
29. Herve CA, Wait R, Venables PJ: **Glucose-6-phosphate isomerase is not a specific autoantigen in rheumatoid arthritis.** *Rheumatology* 2003, **42:**986-988.
30. Matsumoto I, Lee DM, Goldbach-Mansky R, Sumida T, Hitchon CA, Schur PH, Anderson RJ, Coblyn JS, Weinblatt ME, Brenner M, Duclos B, Pasquali JL, El-Gabalawy H, Mathis D, Benoist C: **Low prevalence of antibodies to glucose-6-phosphate isomerase in patients with rheumatoid arthritis and a spectrum of other chronic autoimmune disorders.** *Arthritis Rheum* 2003, **48:**944-954.
31. Rowley M, Tait B, Mackay IR, Cunningham T, Phillips B: **Collagen antibodies in rheumatoid arthritis. Significance of antibodies to denatured collagen and their association with HLA-DR4.** *Arthritis Rheum* 1986, **29:**174-184.
32. Verheijden GF, Rijnders AW, Bos E, Coenen-de Roo CJ, van Staveren CJ, Miltenburg AM, Meijerink JH, Elewaut D, de Keyser F, Veys E, Boots AM: **Human cartilage glycoprotein-39 as a candidate autoantigen in rheumatoid arthritis.** *Arthritis Rheum* 1997, **40:**1115-1125.
33. Li NL, Zhang DQ, Zhou KY, Cartman A, Leroux JY, Poole AR, Zhang YP: **Isolation and characteristics of autoreactive T cells specific to aggrecan G1 domain from rheumatoid arthritis patients.** *Cell Res* 2000, **10:**39-49.
34. Goldbach-Mansky R, Lee J, McCoy A, Hoxworth J, Yarboro C, Smolen JS, Steiner G, Rosen A, Zhang C, Menard HA, Zhou Z J, Palosuo T, Van Venrooij WJ, Wilder RL, Klippel JH, Schumacher HR Jr, El-Gabalawy HS: **Rheumatoid arthritis associated autoantibodies in patients with synovitis of recent onset.** *Arthritis Res* 2000, **2:**236-243.
35. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, van Venrooij WJ: **The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide.** *Arthritis Rheum* 2000, **43:**155-163.
36. van Boekel MA, Vossenaar ER, van den Hoogen FH, van Venrooij WJ: **Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value.** *Arthritis Res* 2002, **4:**87-93.
37. Asaga H, Ishigami A: **Protein deimination in the rat brain after kainate administration: citrulline-containing proteins as a novel marker of neurodegeneration.** *Neurosci Lett* 2001, **299:**5-8.
38. Wahle M, Pierer M, Krause A, Kolker S, Baerwald CG: **Decreased catecholamine-induced cell death in B lymphocytes from patients with rheumatoid arthritis.** *Ann NY Acad Sci* 2002, **966:**425-428.
39. Shimaoka Y, Attrep JF, Hirano T, Ishihara K, Suzuki R, Toyosaki T, Ochi T, Lipsky PE: **Nurse-like cells from bone marrow and synovium of patients with rheumatoid arthritis promote survival and enhance function of human B cells.** *J Clin Invest* 1998, **102:**606-618.
40. Lopez-Hoyos M, Marquina R, Tamayo E, Gonzalez-Rojas J, Izui S, Merino R, Merino J: **Defects in the regulation of B cell apoptosis are required for the production of citrullinated peptide autoantibodies in mice.** *Arthritis Rheum* 2003, **48:**2353-2361.
41. Hill JA, Southwood S, Sette A, Jevnikar AM, Bell DA, Cairns E: **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:**538-541.
42. Wagner UG, Kurtin PJ, Wahner A, Brackertz M, Berry DJ, Goronzy JJ, Weyand CM: **The role of CD8+ CD40L+ T cells in the formation of germinal centers in rheumatoid synovitis.** *J Immunol* 1998, **161:**6390-6397.
43. Schroder AE, Greiner A, Seyfert C, Berek C**: Differentiation of B cells in the nonlymphoid tissue of the synovial membrane of patients with rheumatoid arthritis.** *Proc Natl Acad Sci USA* 1996, **93:**221-225.
44. Krenn V, Schalhorn N, Greiner A, Molitoris R, Konig A, Gohlke F, Muller-Hermelink HK: **Immunohistochemical analysis of proliferating and antigen-presenting cells in rheumatoid synovial tissue.** *Rheumatol Int* 1996, **15:**239-247.
45. Kim HJ, Krenn V, Steinhauser G, Berek C: **Plasma cell development in synovial germinal centers in patients with rheumatoid and reactive arthritis.** *J Immunol* 1999, **162:**3053-3062.
46. Weyand CM, Goronzy JJ: **Ectopic germinal center formation in rheumatoid synovitis.** *Ann NY Acad Sci* 2003, **987:**140-149.
47. Voswinkel J, Weisgerber K, Pfreundschuh M, Gause A: **B lymphocyte involvement in ankylosing spondylitis: the heavy chain variable segment gene repertoire of B lymphocytes from germinal center-like foci in the synovial membrane indicates antigen selection.** *Arthritis Res* 2001, **3:**189-195.
48. Magalhaes R, Gehrke T, Souto-Carneiro MM, Kriegsmann J, Krenn V: **Extensive plasma cell infiltration with crystal IgG inclusions and mutated IgV(H) gene in an osteoarthritis patient with lymphoplasmacellular synovitis. A case report.** *Pathol Res Pract* 2002, **198:**45-50.
49. Shiokawa S, Matsumoto N, Nishimura J: **Clonal analysis of B cells in the osteoarthritis synovium.** *Ann Rheum Dis* 2001, **60:** 802-805.
50. Takemura S, Braun A, Crowson C, Kurtin PJ, Cofield RH, O'Fallon WM, Goronzy JJ, Weyand CM: **Lymphoid neogenesis in rheumatoid synovitis.** *J Immunol* 2001, **167:**1072-1080.
51. Luther SA, Bidgol A, Hargreaves DC, Schmidt A, Xu Y, Paniyadi J, Matloubian M, Cyster JG: **Differing activities of homeostatic chemokines CCL19, CCL21, and CXCL12 in lymphocyte and dendritic cell recruitment and lymphoid neogenesis.** *J Immunol* 2002, **169:**424-433.
52. Weyand CM, Braun A, Takemura S, Goronzy JJ: **Lymphoid microstructures in rheumatoid synovitis.** *Curr Dir Autoimmun* 2001, **3:**168-187.
53. Seki T, Selby J, Haupl T, Winchester R: **Use of differential subtraction method to identify genes that characterize the phenotype of cultured rheumatoid arthritis synoviocytes.** *Arthritis Rheum* 1998, **41:**1356-1364.
54. Dechanet J, Merville P, Durand I, Banchereau J, Miossec P: **The ability of synoviocytes to support terminal differentiation of activated B cells may explain plasma cell accumulation in rheumatoid synovium.** *J Clin Invest* 1995, **95:**456-463.
55. Hauser AE, Debes GF, Arce S, Cassese G, Hamann A, Radbruch A, Manz RA: **Chemotactic responsiveness toward ligands for CXCR3 and CXCR4 is regulated on plasma blasts during the time course of a memory immune response.** *J Immunol* 2002, **169:**1277-1282.
56. Cassese G, Arce S, Hauser AE, Lehnert K, Moewes B, Mostarac M, Muehlinghaus G, Szyska M, Radbruch A, Manz RA: **Plasma cell survival is mediated by synergistic effects of cytokines and adhesion-dependent signals.** *J Immunol* 2003, **171:**1684-1690.
57. Tan SM, Xu D, Roschke V, Perry JW, Arkfeld DG, Ehresmann GR, Migone TS, Hilbert DM, Stohl W: **Local production of B lymphocyte stimulator protein and APRIL in arthritic joints of patients with inflammatory arthritis.** *Arthritis Rheum* 2003, **48:**982-992.
58. Meffre E, Davis E, Schiff C, Cunningham-Rundles C, Ivashkiv LB, Staudt LM, Young JW, Nussenzweig MC: **Circulating human B cells that express surrogate light chains and edited receptors.** *Nat Immunol* 2000, **1:**207-213.
59. Wang YH, Stephan RP, Scheffold A, Kunkel D, Karasuyama H, Radbruch A, Cooper MD: **Differential surrogate light chain expression governs B-cell differentiation.** *Blood* 2002, **99:**2459-2467.
60. Silverman GJ, Weisman S: **Rituximab therapy and autoimmune disorders: prospects for anti-B cell therapy.** *Arthritis Rheum* 2003, **48:**1484-1492.
61. Nishimoto N, Yoshizaki K, Maeda K, Kuritani T, Deguchi H, Sato B, Imai N, Suemura M, Kakehi T, Takagi N, Kishimoto T: **Toxicity, pharmacokinetics, and dose-finding study of repetitive treatment with the humanized anti-interleukin 6 receptor antibody MRA in rheumatoid arthritis. Phase I/II clinical study.** *J Rheumatol* 2003, **30:**1426-1435.

Correspondence

Dr Gregg Silverman, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0663, USA. Tel: +1 858 534 5439; fax: +1 858 534 5399; e-mail: gsilverman@ucsd.edu