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Arthritis Critically Dependent on Innate Immune System Players

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Summary

K/BxN T cell receptor transgenic mice are a model of inflammatory arthritis, similar to rheumatoid arthritis. Disease in these animals is focused specifically on the joints but stems from autoreactivity to a ubiquitously expressed antigen, glucose-6-phosphate isomerase (GPI). T and B cells are both required for disease initiation, but anti-GPI immunoglobulins (Igs), alone, can induce arthritis in lymphocyte-deficient recipients. Here, we show that the arthritogenic Igs act through both Fc receptors (in particular, FcγRIII) and the complement network (C5a). Surprisingly, the alternative pathway of complement activation is critical, while classical pathway components are entirely dispensable. We suggest that autoimmune disease, even one that is organ specific, can occur when mobilization of an adaptive immune response results in runaway activation of the innate response.

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Introduction

Inflammatory arthridites, in particular rheumatoid arthritis (RA), are an important health problem. As such, they have been the focus of intense investigation, but their etiology and pathogenesis remain very controversial (reviewed in Arend, 1997). For example, there is no consensus on what initiates RA—whether it is primarily an autoimmune response reflecting an aberrancy in immune regulation, an inflammatory response to some microbial or mechanical insult, or a combination of the two. There is also dispute over the major players in RA pathogenesis—whether T cells, B cells, other leukocytes, or some combination of them drives disease. Much of the controversy probably stems from the fact that RA is quite heterogeneous, raising the possibility that what has been classified as one disease entity may actually be a collection of disorders, with similar symptoms resulting from dissimilar causes and courses. Seemingly more homogeneous are the end-stage effector mechanisms, whose commonalities include massive recruitment of neutrophils and macrophages, the implication of inflammatory cytokines like tumor necrosis factor (TNF)-α and interleukin (IL)-1, and the synthesis of a battery of degradative enzymes. It is likely, then, that disparate disease courses converge into a common route of destruction.

As far as end-stage effector mechanisms, an important issue is whether and how Fc receptors (FcRs) and components of the complement network are involved. Both are obvious candidates for linking upstream initiation events to downstream effector activities. Several lines of evidence have implicated FcRs, in particular IgG binding receptors (FcγRs), in RA pathogenesis: FcγRIII was detected on synovial intima in normal and arthritic human joints and on invading macrophages in the latter (Edwards et al., 1997); an FcγRIII gene polymorphism has been correlated with human RA susceptibility (Nieto et al., 2000); mice lacking FcRγ (and thereby FcγRI, FcγRIII, and FcεRI) were not susceptible to arthritis induction upon injection of collagen or adjuvant (Kleinau et al., 2000; van Lent et al., 2000); and lack of the inhibitory receptor FcγRIIB was found to exacerbate collagen-induced arthritis (CIA) in susceptible mouse strains

(Kleinau et al., 2000) or permit its induction in normally resistant strains (Yuasa et al., 1999). The complement network was initially implicated in human RA, indirectly, by the colocalization of C3 fragments with immune complexes in joint tissue (Cooke et al., 1975), and by the demonstration that complement activity, as well as early-acting components (C2, C4), is routinely depressed in synovial fluid of patients (reviewed in Zvaifler, 1973). More recently, more direct evidence of complement activation in arthritic joints has been reported (Jose et al., 1990). In murine models of RA, especially CIA, C5 deficiency has frequently been correlated with disease resistance (Wang et al., 1995, 2000), although this has not always been the case (Andersson et al., 1991). Thus, there is evidence implicating both FcRs and the complement network in RA. Their potential inputs are numerous. Concerning the complement pathway, roles in tissue destruction (via C5b-9), in mobilizing inflammatory and synovial cells (via C3a, C4a, or C5a), or in promoting phagocytosis of immune complexes (through CR1 or CR3) are all possible (reviewed in Ravetch and Clynes, 1998). Concerning FcRs, induction of phagocytosis, recruitment and activation of neutrophils and synoviocytes, and amplification of antigen presentation seem most probable (reviewed in Ravetch and Clynes, 1998).

At this point, it is important to delve more deeply: which particular FcRs and complement components are involved in RA? Where do they intervene in the disease process? What is the relationship between these two effector arms? This latter question is of particular interest given the recent suggestion that only one or the other of these arms usually dominates any given inflammatory response (Ravetch and Clynes, 1998).

We have chosen to examine these issues in K/BxN T cell receptor (TCR) transgenic (tg) mice, a recently developed model of inflammatory arthritis (Kouskoff et al., 1996; Korganow et al., 1999; Matsumoto et al., 1999). All K/BxN animals spontaneously develop an autoimmune disease with most (although not all) of the clinical, histological, and immunological features of RA in humans. The murine disorder, critically dependent on both T and B cells, is joint specific but is initiated, then perpetuated, by T, then B, cell autoreactivity to a ubiquitously expressed antigen, GPI. Strikingly, transfer of serum (or purified anti-GPI Igs) from arthritic K/BxN mice into healthy animals provokes arthritis within days, even when the recipients are devoid of lymphocytes. A likely scenario is that GPI:anti-GPI immune complexes (ICs) are the link between the systemic T and B lymphocyte autoreactivity characteristic of K/BxN mice and the ensuing joint-specific destruction. ICs may be differentially generated or retained in the joint, where they engage FcRs and/or activate the complement network, setting off a cascade of events that includes the recruitment and activation of inflammatory cells and synoviocytes, massive production of growth factors and cytokines (in particular TNF-α and IL-1), and the synthesis of degradative enzymes. The relevance of the K/BxN model to human RA is supported by a recent report that serum from almost two-thirds of RA patients contained anti-GPI antibodies (Abs), absent from serum of normal individuals or of patients with Lyme arthritis or Sjogren's syndrome (Schaller et al., 2001).

Here, we establish a critical role for both FcRs and the complement network during the effector phase of inflammatory arthritis. More specifically, we define FcγRIII as the critical IgG receptor, the alternative pathway as the upstream initiator of complement activation, and C5a:C5aR-mediated interactions as the downstream complement effector.

Results

The K/BxN serum-transfer system is highly advantageous for studying the effector mechanisms that link the production of potentially pathogenic Igs and the overt development of arthritis. Disease induction in this system is rapid, robust, and reproducible (Korganow et al., 1999) and contrasts with most murine models of autoimmune disease in being applicable in a number of mouse strains (Ji et al., 2001). This last feature is a particular attraction when screening for the effects of diverse natural and engineered mutations.

A Role for FcRs

Given that the arthritogenic activity of K/BxN serum resides solely in the IgG fraction (Korganow et al., 1999), we focused on the role of Fc receptors for this isotype in serum-induced disease. Both high-affinity, FcγRI, and low-affinity, FcγRIII, employ the common γ chain, FcRγ (as does FcϵRI). Therefore, as a first look, we evaluated the effect of the FcRγ null mutation (Takai et al., 1994). Serum from arthritic K/BxN mice was injected into FcR$\gamma^{-/-}$ recipients and control littermates, and diverse disease parameters were followed over time (Figures 1A and 1B). Arthritis arose in all control animals, any heterogeneity almost certainly reflecting their mixed (B6x129/Sv) genetic background. In contrast, there was no evidence of arthritis in the FcRγ-deficient animals for any parameter, including histological score.

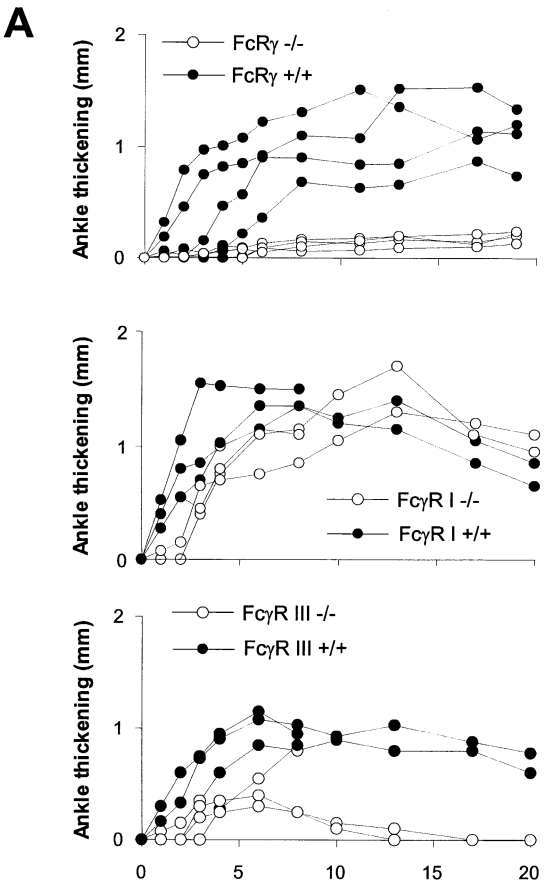
To assess the relative importance of the two receptors for IgG, we evaluated the effect of FcγRI- and FcγRIII null mutations (Hazenbos et al., 1996) (Figures 1A and 1B). Lack of FcγRI appeared to have no influence on arthritis development. All FcγRIII-deficient mice succumbed to arthritis, but it was attenuated vis-à-vis the disease in wild-type controls according to all parameters: the day of onset was delayed, ankle thickening was reduced, and histological analysis revealed less infiltration and cartilage destruction.

In a separate study (Ji et al., 2001), we tested the influence of the inhibitory receptor FcγRIIB. Neither the FcγRIIB null mutation (Takai et al., 1996) on a mixed B6x129/Sv genetic background nor a naturally occurring defective FcγRIIB allele (Luan et al., 1996) placed on a B6 background affected development of K/BxN serum-transferred arthritis.

In short, Fc receptors are required for efficient induction of arthritis upon transfer of K/BxN serum. FcγRI plays no essential role, and FcγRII appears not to have an inhibitory influence, at least in the genetic contexts so far examined. Intriguingly, the importance of FcγRIII, although clearly evidenced, is markedly less than that of the common FcRγ chain.

A Role for the Complement Network

To address the role of the complement network in K/BxN serum-transferred arthritis, we initially focused on C5 for



B

Days after serum transfer

Strain	Incidence	Day of onset	Max. AT(mm)	Hist. Score
FcRγ-/-	0/10	-	0,10	1.0
FcRγ+/+	6/6	1,1,3,5,2,3	1.28	6.7
FcγR I-/-	6/6	1,1,1,3,2,2	1.31	9.0
FcγR I+/+	5/5	1,1,1,1,1	1.37	9.0
FcγR III-/-	5/6	-,2,3*,3*,3*,7	0.42	4.0
FcγR III+/+	5/5	2,2,2,2,2	1.16	9.0

Figure 1. Role of Fc Receptors in Arthritis Induced by K/BxN Serum Transfer

FcR-deficient and control mice (matched for genetic background) were injected with 150 μl serum from arthritic K/BxN animals on days 0 and 2. Arthritis was evaluated by measuring clinical index and ankle thickening (see Experimental Procedures).

(A) Data from representative experiments, each curve signifying an individual mouse.

(B) Tabulation of the results for six mice of each line. *, very weak arthritis in FcγRIII mice, CI of 0.5 to 1; MaxAT, maximum increase in ankle thickness (mm). The histological score sums scores from knee, ankle, and tarsal joints (1, minimum synovial hyperplasia; 2, limited inflammatory infiltration; 3, massive infiltration; 4, massive infiltration with cartilage and bone destruction); maximum score = 12.

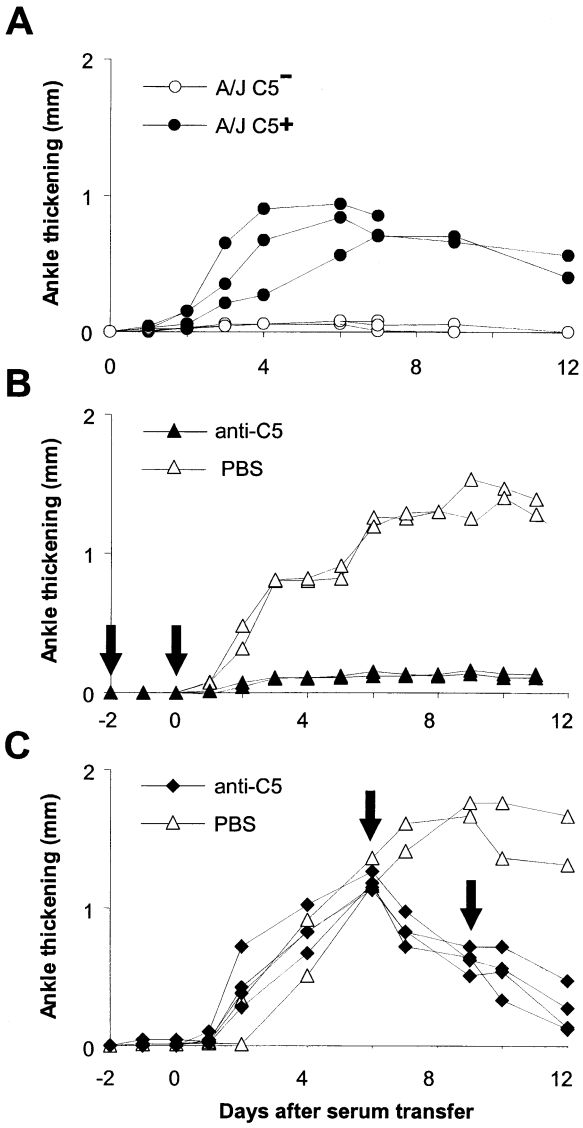


Figure 2. Role of the Complement Network

(A) A/J strain mice or a C5-proficient congenic variant was injected with K/BxN serum and arthritis followed by measuring the increase in ankle thickness. Each curve represents an individual mouse. These data represent animals scored in the same experiment and are a subset of those in Figure 3.

(B) B6 mice were injected with K/BxN serum (d0, d2) with or without pretreatment with anti-C5 mAb (d−2, d0; arrows), and the development of arthritis followed as above.

(C) As in (B), except that the anti-C5 mAb treatment was initiated at d5, when arthritis was already evident.

two major reasons. First, C5 is pivotal in the complement network, both effector pathways leading from it and the three initiating pathways leading into it. Second, the critical reagents needed for such a study were available, both a C5-deficient mouse strain (Gervais et al., 1989) and an anti-C5 monoclonal antibody (mAb) (Frei et al., 1987b).

K/BxN serum was injected into C5-deficient and C5-sufficient A/J congenic mice, and signs of arthritis were monitored over time (Figure 2A). Mice lacking C5 showed no signs of disease development. Since the C5-deficient strain was a congenic rather than a knockout variant, it was necessary to confirm that the lack of response to arthritogenic serum was due to the defective C5 gene itself and not to some adjacent genetic

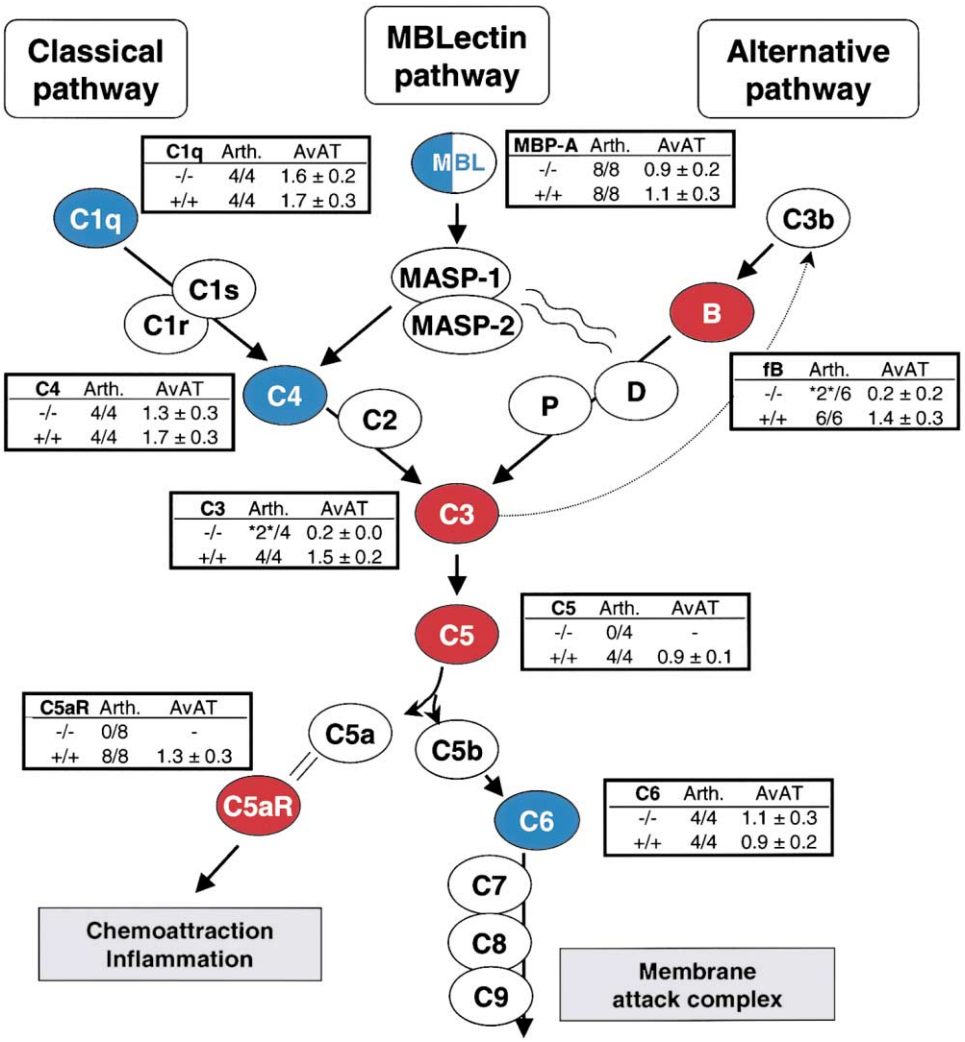


Figure 3. The Alternative, Not the Classical, Pathway of Complement Activation Is Involved in K/BxN Serum-Transferred Arthritis

The three initiating pathways leading into C5 and the two effector pathways leading from them are schematized. Tabulated next to a particular component is the effect of its deficiency, assessed for each case in at least two independent experiments, each with at least two individual mutant mice. Scoring was as described in Experimental Procedures. Arth. represents the proportion of affected mice, AvAT the average max ankle swelling. Absence of arthritis corresponds to no clinical signs at all. Blue ovals signify no effect; red ovals reflect significant inhibition. MBL is shown as half-blue/half-white because an MBP-A deficiency showed no effect, but an MBP-C deficiency has not yet been tested. The asterisks for C3 and fB indicate very weak disease manifestations.

variation. Therefore, recipients of K/BxN serum were treated with anti-C5 mAb starting from 2 days before serum injection (Figure 2B). These animals also showed no signs of disease. Interestingly, anti-C5 mAb treatment could also reverse ongoing disease when injected several days after arthritis onset (Figure 2C).

Thus, the complement pathway is a critical player in K/BxN serum-induced arthritis.

It's the Alternative Pathway!

Next, we were interested in defining the upstream and downstream pathways responsible for the arthritogenicity of K/BxN serum, i.e., the route leading from and into C5. The approach was to monitor the effect of injecting serum from arthritic K/BxN mice into various genetically engineered or naturally variant recipients deficient for a particular complement pathway component, always being careful to compare control animals that were the most closely genetically matched possible. The data are summarized and oriented within the complement network in Figure 3; results from representative individual experiments for each mutant strain are presented in Figure 4A.

Two effector pathways lead from C5 (Figure 3). First, its cleavage product C5b initiates formation of the membrane attack complex. This pathway was not required for K/BxN serum-transferred arthritis because a C6 deficiency did not influence disease progression (Figures 3 and 4). The other C5 cleavage product, C5a, is a potent promoter of inflammation, having strong chemotaxic properties, in particular for neutrophils, as well as a plethora of other proinflammatory activities. This effector pathway was crucial for K/BxN serum-transferred arthritis, as a null mutation of the C5a receptor (C5aR)

A

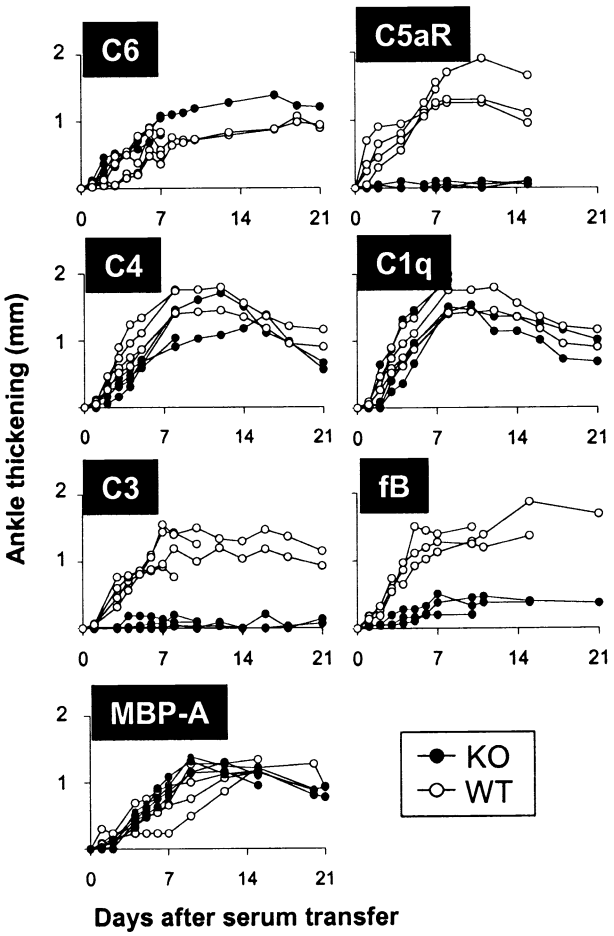


Figure 4. Role of Individual Complement Components

(A) Mice deficient in particular components of the complement network (and genetically matched controls) were injected with K/BxN serum, and the development of arthritis monitored as in Figure 1. Curves signify individual mice in representative experiments. Data pooled from multiple experiments appear in the mini-tables of Figure 3.

(B) Pooled data for mice lacking particular complement receptors.

B

Strain	Incidence	Day of onset	AvAT (mm)±SD	Hist. Score
CR1/2-/-	5/5	1,1,1,2,3	1.0 ± 0.3	7.5
CR1/2+/+	5/5	2,2,2,2,2	0.90 ± 0.24	9.0
CR3-/-	5/5	1,1,2,2,3	0.92 ± 0.35	9.0
CR3+/+	5/5	2,2,2,2,2	0.90 ± 0.24	9.0

(Hopken et al., 1996) completely abrogated disease development (Figures 3 and 4). The inhibitory effect of the C5aR deficiency was as potent as that observed with a C5 mutation (compare with Figure 2A, top panel): no clinical or histological abnormalities were detected in either case, suggesting that all downstream effector activity channels through C5a:C5aR interactions and the inflammation that ensues.

Three initiator pathways lead into C5: the classical and alternative pathways feed directly into C3, while the mannose binding (MB) lectin pathway channels indirectly, certainly by the classical and perhaps by the alternative pathway, the juncture of the latter being presently unknown (Schweinle et al., 1989) (Figure 3). The importance of the classical pathway was assessed by injecting K/BxN serum into mice lacking C4 (Wessels et al., 1995). These animals developed disease as usual, arguing against a required role for the classical pathway (Figures 3 and 4). Given that this result was quite unexpected, we confirmed it by two means. First, we eliminated the possibility that components in the injected serum were complementing the C4 deficiency by transferring purified IgG, rather than serum, from arthritic K/BxN donors: recipients lacking C4 developed arthritis with the standard characteristics (data not shown). Second, we tested mice with a null mutation in the gene encoding an upstream component, C1q (Botto et al., 1998): these animals also became arthritic upon serum injection (Figures 3 and 4).

The dispensability of the classical pathway raised the

question of whether C5 might not be directly cleaved by proteases derived from invading inflammatory cells, in particular neutrophils, as has been described in some contexts (Wetsel and Kolb, 1983). If this notion were true, serum-induced disease would be C3 independent. This turned out not to be the case: there was a strong inhibition of arthritis development in C3 null mice (Wessels et al., 1995) (Figures 3 and 4), although, interestingly, it was not as profound a block as was seen in C5aR-deficient animals. Unlike the latter strain, the former showed mild swelling in the occasional paw, as well as sporadic histological signs such as synovial hyperplasia and infiltration.

To assess the contribution of the alternative pathway, we analyzed disease induction in mice devoid of factor B (Watanabe et al., 2000). Most of these animals did not develop arthritis upon K/BxN serum transfer, although a few did show some weak clinical and histological signs—occasional joint swelling, synovial hyperplasia, and sparse leukocyte infiltrates (Figures 3 and 4). This distinctly muted response was reminiscent of that observed with C3 null animals.

The critical issue then became what activates/amplifies the alternative pathway. A major mechanism is thought to be amplification via C3b fragment generated through the classical pathway (Watanabe et al., 2000). Yet, as detailed above, we have already eliminated any essential role for classical pathway constituents. Another mechanism of activating the alternative pathway is via mannose binding protein (MBP) in the MBLectin pathway, either the MBP-A constituent, MBP-C, or both. This has been suggested (Schweinle et al., 1989) but is controversial, and details of how the MBLectin and alternative pathways might link remain unknown. Mice harboring a null mutation at the locus encoding MBP-A have recently been generated, but an MBP-C mutant is not yet available. Lack of MBP-A had no apparent effect on serum-induced disease (Figures 3 and 4), leaving open the possibilities that MBP-C or either one of the two plays a required role, or that the MBLectin pathway is not involved.

A related issue was whether and which complement receptors (CRs) might be involved in K/BxN serum-transferred arthritis. CR1 binds the C3b, iC3b, and C4b fragments; CR2, the C3d and iC3b fragments; and CR3, the iC3b fragment. These interactions have been implicated in immune adherance of opsonized particles, phagocytosis, IC clearance, and signal transduction. As illustrated in Figure 4B, neither a combined deficiency in CR1 and CR2 (Molina et al., 1996) nor a deficit in CR3 (Coxon et al., 1996) had a detectable effect on serum-induced disease.

In summary, the critical role of the complement network in K/BxN serum-transferred arthritis is initiated via the alternative pathway and effected through C5a:C5aR interactions. Factor B, C3, C5, and C5aR all have important influences on disease induction, whereas C1q, C4, MBP-A, and C6 and CRs 1, 2, and 3 are all dispensable.

Integration of the FcR and Complement Network Influences

Several scenarios could account for the dual importance of FcRs and the complement network in K/BxN arthritis, invoking independently required roles or critical roles in series. Important clues to how these influences might be integrated could come from delineating when precisely they impinge on disease progression or from identifying partial or transient phenotypes in their absence.

First, we applied a recently developed in vivo imaging strategy to visualization of the early stages of serum-transferred arthritis. This approach, already applied in neoplastic settings, relies on protease-activated near infra-red fluorescent (NIRF) probes to detect the enhanced endocytic/phagocytic and protease activities characteristic of infiltrating leukocytes (e.g., macrophages, dendritic cells, neutrophils) (Weissleder et al., 1999). Fluorochrome tags are conjugated to a protected graft copolymer at high density, resulting in their quenching; also incorporated into the copolymer are recognition sites for cathepsin B; cleavage at these sites releases the tags, permitting imaging via external illumination since both the excitation and emission wavelengths of the fluorochrome can traverse soft tissue. Mice were iv-injected with this probe and visualization performed by NIRF reflectance imaging. As illustrated in Figure 5A, for a 60-day-old K/BxN TCR tg mouse, strong fluorescence could be visualized over the ankle and some of the digits in arthritic animals, while such a signal was not detectable in nonarthritic controls. Clear fluorescence was also detected, albeit not as strongly, when wild-type mice were injected with K/BxN serum; a signal could be observed 20 hr after administration of serum, at a time when the outward clinical manifestations were still very discrete (Figure 5B). Only the wild-type mice showed a response to K/BxN serum injection in this assay; no significant signal (quantitated in Figure 5C) was detected in either the C5- or FcRγ-deficient mice. This result indicates that FcRs and the complement network are both required for the earliest recruitment of inflammatory cells to the lesion and/or their activation.

Second, we assessed the local consequences of complement activation via immunohistology, testing for complement deposition by staining joint sections with anti-C3 reagents. As shown in Figure 6A, the arthritic lesion in wild-type recipients of K/BxN serum is accompanied by C3 deposits in several areas of the joint—in the area of proliferative synovitis and very strikingly aligned along the cartilage surface. These correlate with IgG deposits at the same sites, evidenced in costaining experiments (Figure 6A). In FcRγ-deficient mice (Figure 6B), the C3 deposits were largely absent, although in three of the four animals examined, we noted small patches of mild but significant complement deposition. IgG deposits were also present, albeit in reduced amounts. In C5-deficient mice (Figure 6B), the C3 and IgG deposits were completely absent. The conclusion from this analysis is that both FcRs and the complement network are required for cell recruitment/activation events at the earliest stages of arthritis but that complement is necessary and at least partially sufficient for the generation of the molecular aggregates that provoke these events.

Discussion

We have exploited the K/BxN serum transfer system to focus on end-stage effector mechanisms in inflamma-

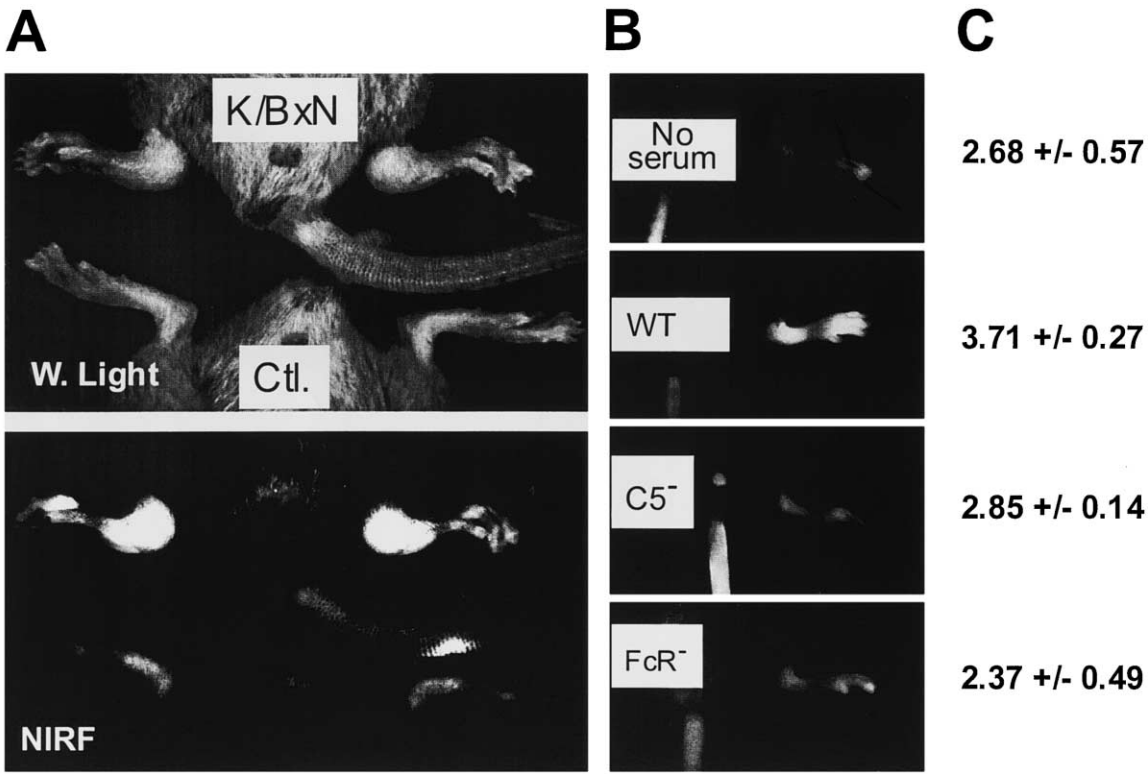


Figure 5. NIRF Imaging of Inflammation

(A) An arthritic K/BxN TCR tg mouse and a control littermate were injected with quenched NIRF probe. Probe cleavage by inflammatory cathepsins results in increased fluorescence in the arthritic joint of the K/BxN mouse.

(B) Relative fluorescence intensity 24 hr after transfer of K/BxN serum into wild-type or C5-deficient or FcR-deficient mice.

(C) Average signal intensity (log_{10} scale) over the ankle area, averaged from three to four mice per group.

tory arthritis. In particular, we addressed the role of FcRs and components of the complement network in linking the production of potentially pathogenic Igs and the development of joint lesions. Several important findings emerged: (1) that both effector mechanisms are required for serum-transferred arthritis; (2) that the critical role of FcγRs is mediated largely, though perhaps not entirely, through FcγRIII; (3) that activation of the complement network is initiated via the alternative pathway, the classical pathway being entirely dispensable; and (4) that the proinflammatory sequelae of C5a:C5aR interactions are key to the complement pathway's involvement.

Both FcRs and the Complement Network

Somewhat surprising is the finding that FcRs and the complement network are equally indispensable for K/BxN serum-transferred arthritis. It has been argued that these two effector arms play distinct roles in immune responses, the former primordial in inflammatory reactions elicited by Abs or ICs, the latter in innate reactions to bacterial pathogens and toxins (Ravetch and Clynes, 1998). However, it now seems clear that both arms can have an important function in Ab/IC-induced inflammation (Kohl and Gessner, 1999; Ravetch and Bolland, 2001), their relative contributions varying with the particular tissue(s) involved and the genetic background. Anti-GPI-induced arthritis would appear to be on one end of the spectrum, both effector arms being absolutely necessary. Perhaps the explanation for this dual requirement lies in the complex and chronic nature of arthritis, dependent on mobilization of multiple effector cell types, cyto/chemokines, and degradative processes. It is not yet clear whether the two effector arms provide independent required inputs or whether they make their essential inputs in series. Also not clear is what needed element(s) is (are) uniquely mobilized through FcRs versus components of the complement network.

FcRs

That the K/BxN serum-transfer system is dependent on FcRs fits well with past indications in other animal models of RA and in human RA patients. This study brings two important additional findings. First, FcRs play a critical role during the final effector phase of disease. It was possible that FcRs were required for antigen presentation events associated either with the breakdown of tolerance to self-Ags or with the initiation of an anti-self-Ag immune response that culminated in an inflammatory reaction (Ravetch and Bolland, 2001). This remains an added possibility in the K/BxN model but is certainly not the only role. In this context, FcRs appear to be a link between GPI:anti-GPI complexes and downstream inflammatory mechanisms.

Second, we define the dominant FcR as FcγRIII. This receptor is expressed on mast cells, neutrophils, macrophages, and NK cells. Its engagement is known to activate mast cells, neutrophils, and macrophages; recruit

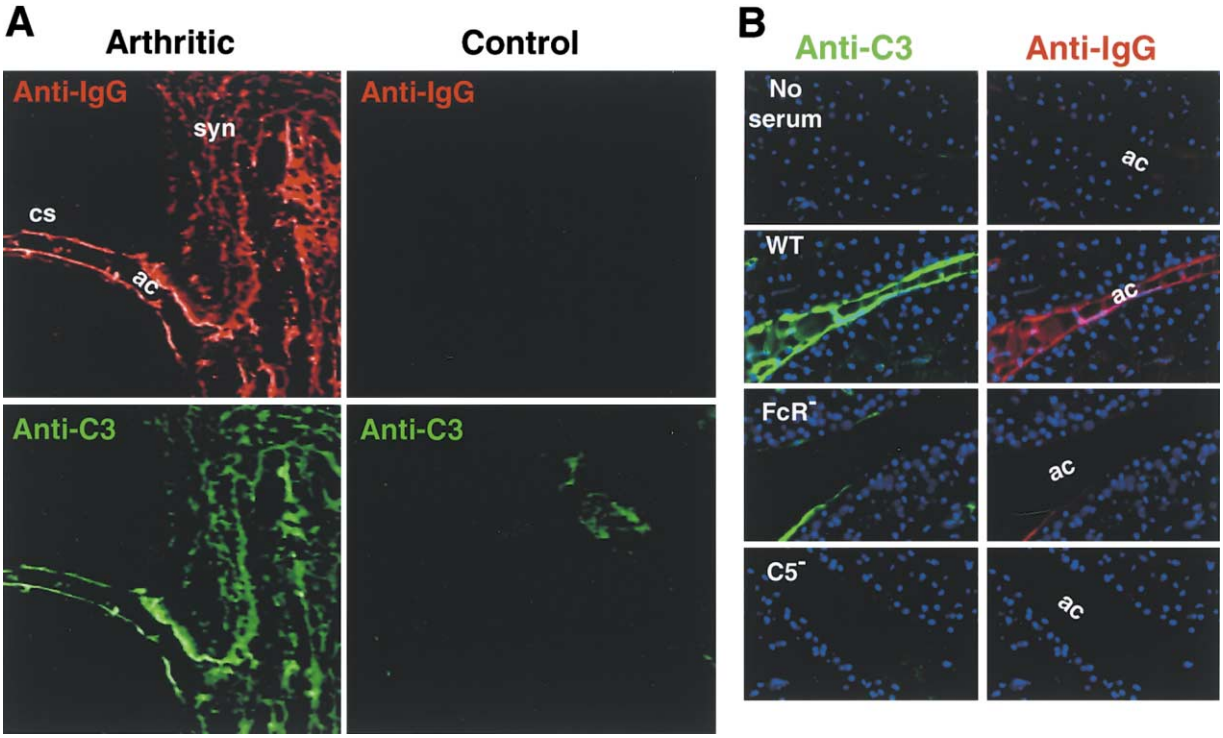


Figure 6. C3/IgG Deposits

Ankle cryostat sections from B6 mice (injected a week previously with K/BxN or control serum) were stained with anti-C3 (green) and anti-IgG (red). Note the deposits, absent in the control mouse, on the cartilage surface (cs) and synovial tissue (syn); ac, articular cavity.
(B) Deposits along the cartilage surface of wild-type or C5-deficient or FcR-deficient mice injected with K/BxN serum (sectioning and staining as in [A]).

(or tether) neutrophils and macrophages to (at) the site of inflammation; induce secretion of TNF-α, IL-1, other cytokines, and chemokines; and induce release of lysosomal enzymes, oxygen radicals, and vasoactive substances (Ravetch and Bolland, 2001). These activities render FcγRIII an (if not the) orchestrator of the Arthus reaction; they can also be readily integrated into a scenario of arthritis development in the K/BxN model, in keeping with the dominant role of IgG1 (our unpublished data). Intriguingly, polymorphisms in the gene encoding FcγRIIIB in humans have recently been tied to RA susceptibility (Nieto et al., 2000).

In the Arthus reaction and in other contexts, signals transmitted through FcγRIII are attenuated by FcγRII-mediated signals (reviewed in Ravetch and Bolland, 2001). Thus, the lack of influence of FcγRII mutations, null or otherwise (Ji et al., 2001), is somewhat surprising. It is possible that an influence will be observed on a different genetic background or that this particular inflammatory reaction is just not sensitive to FcγRII-mediated signals, both explanations having precedents (Bolland and Ravetch, 2000; Schiller et al., 2000).

Yet FcγRIII seems not to be the whole story in the K/BxN model because the absence of this receptor did not completely prevent disease, while mice lacking the common chain, FcRγ, were completely resistant. Another receptor that depends on the common γ chain, not necessarily FcγRI or FcϵRI but perhaps a new member of the FcR family (Davis et al., 2001; Hatzivassiliou et al., 2001), may play a role.

The Complement Network

That the complement network is critical for K/BxN serum-transferred arthritis is again consistent with existing data on human RA patients and murine RA models. New here is the definition of the effector pathway leading from C5. It seems that the membrane attack complex, seeded by C5b, does not play a required role; in contrast, the anaphylatoxin effects unleashed by C5a:C5aR binding are critical. These encompass a multitude of activities: vasopermeation and vasodilation; chemotaxis of several cell types—notably, mast cells, neutrophils, and macrophages; degranulation of basophils and mast cells; stimulation of respiratory burst by several cell types; and induction of inflammatory cyto/chemokine release (Gerard and Gerard, 1994). Given that C3a is also a by-product of complement network activation via all three initiating pathways, is upstream of C5a, and is also an anaphylatoxin with many of the same properties, it is surprising that deficiencies in C5 or C5aR have such drastic effects. Perhaps the explanation lies in the inability of C3a, and the potent ability of C5a, to attract neutrophils to inflammatory sites and activate them (Daffern et al., 1995). Neutrophils are among the earliest participants in the joint lesion provoked by K/BxN serum transfer and, in their absence, no lesion develops (Wipke and Allen, 2001); we will argue below that they are critical inflammation amplifiers. Consistent with such a role is the fact that depletion of neutrophils reverses ongoing disease (Wipke and Allen, 2001), as does mAb blockade of C5.

Also new is the demonstration that the route of initiation is the alternative pathway, while the classical pathway is entirely dispensable. This finding, though initially surprising, is consistent with the fact that the dominant isotype of anti-GPI Abs in K/BxN mice is IgG1 (Kouskoff et al., 1996) and that a pool of IgG1 anti-GPI mAbs is capable of inducing arthritis in lymphocyte-deficient recipients (M. Maccioni et al., submitted), murine IgG1 being very poor at complement activation via the classical pathway. In another mouse model of systemic autoimmunity, the MRL/lpr strain, a mutation of factor B led to a drastic reduction in development of disease, but the involvement of the classical pathway was not tested, and it was assumed that the alternative pathway merely served to amplify a response initiated via the classical route (Watanabe et al., 2000). In light of our results, it is important to experimentally verify this assumption.

What initiates the alternative pathway in the K/BxN serum-transfer system—a pathway generally thought to be focused on microbial surfaces? The possibility we favor is that mobilization of the alternative pathway is through formation and stabilization of surface-bound C3b fragments. C3 circulating in the serum is constitutively cleaved at low levels into C3a and C3b, in the latter case revealing a reactive thioester that permits covalent attachment to proteins in the vicinity. Free C3b and C3b-decorated proteins are normally of very short half-life due to inactivation by factors H and I. However, C3b can bind to IgG ICs to form $C3b_2$-IgG complexes that will bind to surfaces, cluster into lattices, and support assembly of C3 and C5 convertases (Vivanco et al., 1999). Joining in the $C3b_2$-IgG complex is properdin, whose binding induces the participation of factor B, thereby activating the alternative pathway; properdin also enhances the C3 convertase activity of the assembling complex (Jelezarova et al., 2000; Schwaeble and Reid, 1999). That neutrophils are the major producers of properdin may explain why C5a:C5aR interactions are so critical in the K/BxN serum-transfer model (Figures 5B and 5C): C5a has potent neutrophil chemotaxic and degranulation activities (unlike C3a), and neutrophils, by producing properdin (as well as additional C3 and factor B), can strongly amplify complement activation via the alternative pathway (Schwaeble and Reid, 1999). C5a-promoted inflammation would thus amplify the deposition and stabilization of $C3b_2$-IgG-GPI complexes; without this amplification, complexes would be cleared, explaining why no C3-IgG deposits were seen in the absence of C5.

As mentioned above, involvement of the complement network in arthritis had generally been assumed to reflect the classical pathway of activation. The one exception is juvenile rheumatoid arthritis, where several reports have provided evidence for mobilization of the alternative pathway and have argued against the importance of the classical (e.g., Aggarwal et al., 2000). It is imperative to revisit this issue in other subsets of RA patients, especially with the more performant tools now at our disposal.

Conclusion

K/BxN serum-induced arthritis is mediated by anti-GPI IgGs (Korganow et al., 1996; Matsumoto et al., 1999). Here, we demonstrate that development of this disease requires both FcγRIII and C5aR. It has also been shown to need neutrophils (Wipke and Allen, 2001), mast cells (D.L., C.B., and D.M., unpublished data), and inflammatory cytokines such as IL-1 and TNF-α (H.J., K.O., C.B., and D.M., unpublished data). These dependencies are highly evocative of the Arthus reaction (Ravetch and Bolland, 2001; Kohl and Gessner, 1999), so it is tempting to propose a disease scenario in such a framework. GPI:anti-GPI complexes accumulate in the joint—in particular, immobilized at the cartilage surface (Figure 6 and above), but also in circulation through synovial tissue (like all tissues [Kouskoff et al., 1996]). On the one hand, as we have discussed, they may bind and stabilize low levels of C3b "ticking over," aggregate into lattices at the cartilage surface, and seed assembly of C3 and C5 convertases, thereby initiating the alternative pathway of complement activation. The major role of the C5a that is generated may be to recruit neutrophils, which would have multiple functions, a critical one being amplification of a complement activation loop feeding through the alternative pathway. On the other hand, GPI:anti-GPI complexes may engage FcRs on cells in the synovial tissue and, as in the Arthus reaction (Sylvestre and Ravetch, 1996; Zhang et al., 1992), FcγRIII engagement on mast cells may permit their almost instantaneous recruitment and degranulation. This would be consistent with many observations of recruitment and activation of mast cells in the lesions of RA patients (e.g., Olsson et al., 2001), with the recent finding that a drug that blocks mast cell degranulation suppresses CIA (Malfait et al., 1999) and with our recent observation that mice lacking mast cells are not prone to K/BxN serum-transferred arthritis (D.L., C.B., D.M., unpublished data). The combined mobilization of these two effector arms would lead to massive chronic inflammation and ultimately to joint destruction. Such a series of events could well take place in other murine arthritis models known to have a critical B cell component—in particular, CIA and antigen-induced arthritis. The relevance to RA, or to particular subsets of it, begs to be assessed.

This scenario reverses our standard view of the relationship between innate and adaptive immunity. Instead of an innate response promoting an adaptive one, as happens in microbial infections, the potent adaptive response to GPI in the K/BxN model recruits and overstimulates the innate immune system. It will be interesting to see how many other autoimmune diseases follow like scenarios.

Experimental Procedures

Mice

The following mice were used for serum transfer at 4–5 weeks of age: $FcR\gamma^{-/-}$ (Takai et al., 1994) on a mixed B6x129 background, control: (B6x129P3/J)F2 (both from the Jackson Laboratory [JAX]); $Fc\gamma RII^{-/-}$ (Takai et al., 1996) on a mixed B6x129 background, control: (B6x129S3/SvImJ)F2 (JAX); $Fc\gamma RIII^{-/-}$ (Hazenbos et al., 1996) on both B6 (N = 10 generations) and (B6x129)F2 backgrounds, control: heterozygous littermates (University Medical Center, Utrecht); $Fc\gamma RI^{-/-}$ on the Balb/c background (J.S.V., unpublished data), control: age/sex-matched Balb/c; $CR1/2^{-/-}$ (Ahearn et al., 1996) and $CR3^{-/-}$ (Coxon et al., 1996) on a mixed B6x129 background, control: (B6x129)F2 (Utrecht); A/J congenic C5-sufficient

(A/J^{-}C5^{+}) and C5-deficient (A/J^{-}C5^{-}) mice (Zal et al., 1994) (National Institute for Medical Research, London); C1q$^{-/-}$ (Botto et al., 1998) on a B6 background (N = 10) (Columbia University), control: B6 (JAX); factor B-deficient (fB$^{-/-}$) (Matsumoto et al., 1997) on a mixed B6x129 background, control: factor B-positive littermates (University of Colorado); C3$^{-/-}$, C4$^{-/-}$, C5aR$^{-/-}$ (Wessels et al., 1995; Fischer et al., 1996; Hopken et al., 1996) on the B6 background (N = 5–7), control: B6 (Harvard Medical School); C6$^{-/-}$ (Orren et al., 1989) on the C3H/He background (University of Wales College of Medicine), control: C3H/HeJ (JAX); MBP-A$^{-/-}$ on a mixed B6x129 background (A.E., unpublished data), control: parallel bred (B6x129)F1 (Massachusetts General Hospital).

Serum Transfer Protocol and Arthritis Scoring

K/BxN serum pools were prepared from arthritic mice at 60 days of age. Arthritis was induced by i.p. injection of 150–200 μl serum at days 0 and 2. A clinical index was evaluated over time (one point for each affected paw; 0.5 points for a paw with only mild swelling/redness or only a few digits affected). Ankle thickness was measured by a caliper (Korganow et al., 1999), ankle thickening being defined as the difference in ankle thickness from the day 0 measure.

Arthritis Inhibition with Anti-C5 mAb

Purified anti-C5 mAb (BB5.1) (Frei et al., 1987a) was purified by protein-G chromatography from tissue culture supernatant. Anti-C5 mAb (1 mg per mouse) was injected at various times relative to K/BxN serum transfer (200 μl per mouse, single injection).

Imaging

In vivo imaging of inflammatory arthritis with a protease-activatable NIRF probe will be described in detail elsewhere (U.M. et al., unpublished data). In brief, the probe includes the Cy5.5 fluorochrome (Amersham, Piscataway, NJ), self-quenched by grafting in multiple copies on a long copolymer consisting of poly-L-lysine sterically protected by multiple methoxypolyethylene glycol side chains. Copolymer cleavage by cathepsin B releases Cy5.5, with a large increase in fluorescence yield. The mouse imaging system consists of a Cy5.5 excitation light source generated by band pass filtering white light (610–650 nm, Omega Optical, Brattleboro, VT) and a recording device consisting of a bandpass emission filter (680–720 nm), a f/1.2, 12.5–70 mm zoom lens, and a scientific grade CCD camera (Kodak, Rochester, NY). Image capture and signal intensity measurements, including region of interest (ROI) measurements, were performed on 1D digital science software (Kodak). At specific times after injection of K/BxN arthritic serum, animals were iv injected with 2 nmol of optical probe and imaged under anesthesia (i.p. ketamine injection) at several subsequent time points. Fluorescent images were acquired for 2 min each, and a corresponding white light image was acquired immediately before for anatomic correlation.

Histology

The basic procedure of fixation, decalcification, paraffin sections, and hematoxylin/eosin staining of joint sections was as described (Kouskoff et al., 1996). For immunohistology, unfixed and undecalcified cryostat sections were obtained by a modified method (Rijntjes et al., 1979). In brief, dissected ankle joints without skin were embedded in OCT, frozen in dry ice isopentane, and mounted on a cryomicrotome support at −25°C. After trimming the tissue block to a desired level, transparent tape (Instrumedics, Inc., Hackensack, NJ) was fastened onto the section surface of the block. Sagittal sections (6 or 8 μm thick) were cut underneath the tape, and the tissue was subsequently transferred to an adhesive-coated slide. Slides were stored at −80°C until use, then acetone-fixed for 30 s to 1 min and air dried for 30 min. The deposition of C3 and IgG was detected by FITC-conjugated goat anti-mouse C3 (ICN/CAPPEL) and Texas red-conjugated goat anti-mouse IgG (Jackson ImmunoResearch). Nuclei were counterstained with 50 ng DAPI (Molecular Probes).

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