

## EDITORIAL

# Systemic Humoral Autoimmunity but Joint-Specific Inflammation: The Syndrome of Rheumatoid Arthritis

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In our ongoing efforts to explain the immunopathogenesis of rheumatoid arthritis (RA), we will integrate and highlight two recent advances. The first advance is the identification of IgG autoantibodies to citrullinated peptides in a substantial (30–50%) portion of patients with RA and their role as a diagnostic marker for RA (1,2). Surprisingly, this antibody binds to a ubiquitous autoantigen, citrullinated peptide, yet leads to a joint-specific disease. The mechanism through which anti-citrullinated peptide antibodies mediate joint disease is unknown. However, clues to their pathogenesis in RA can be deduced from the K/BxN mouse model of arthritis. The second advance, the K/BxN mouse model, features IgG autoantibodies that likewise bind to a ubiquitous self antigen, glucose-6-phosphate isomerase (GPI), resulting in joint-centered pathology. Analogous to immune complex-mediated glomerulonephritis, we propose a disease paradigm in which joints serve as a repository for autoantibodies, culminating in immune complex formation and subsequent immune activation and synovitis.

### The model

K/BxN, an established murine model of arthritis (3–11), is based on the KRN-TCR-transgenic

mouse.\* The KRN T cell receptor (TCR) recognizes bovine RNase peptide bound to the class II major histocompatibility complex (MHC) molecule IA<sup>k</sup>. In the initial H-2<sup>b</sup> background, these mice had no autoimmune phenotype. Remarkably, when the KRN-transgenic mouse was bred to the NOD mouse, all of the F<sub>1</sub> offspring (referred to as K/BxN) spontaneously developed a joint-specific, autoimmune, inflammatory synovitis at age 3–5 weeks (3–5), which faithfully mimicked many clinical, pathologic, and immunologic features of RA. Cartilage destruction and bone erosion occur in the later stages of the disease. By serendipity, the KRN TCR also recognizes a self antigen (GPI) bound to the IA<sup>g7</sup> molecule of the NOD-specific class II MHC. Thus, in K/BxN mice, KRN T cells are autoreactive.

Particularly important for dissecting immunopathologic mechanisms, transfer of serum from K/BxN mice into wild-type (WT) mice results in joint inflammation within a few days (6,7) (Figure 1). Disease is initiated by IgG and can be induced in lymphocyte-deficient recipients, indicating independence of T cells and B cells in this passive-transfer model. Similar to human RA, joint-specific immune complex formation occurs, with subsequent leukocyte recruitment and inflammation (6,7,10) (Table 1). However, the passive antibody-transfer model does not result in pannus formation.

The target antigen recognized in K/BxN mice was identified as GPI (3–5) (Table 2). This ubiquitous glycolytic enzyme catalyzes the interconversion of glucose-6-phosphate and fructose-6-phosphate in the second step of glycolysis. Purified anti-GPI IgG transfers the disease phenotype (6,7). Thus, in this model, KRN T cells recognize GPI and provide help to GPI-specific B cells to produce arthritogenic IgG. With 100% penetrance and rapid onset of the disease, both the sponta-

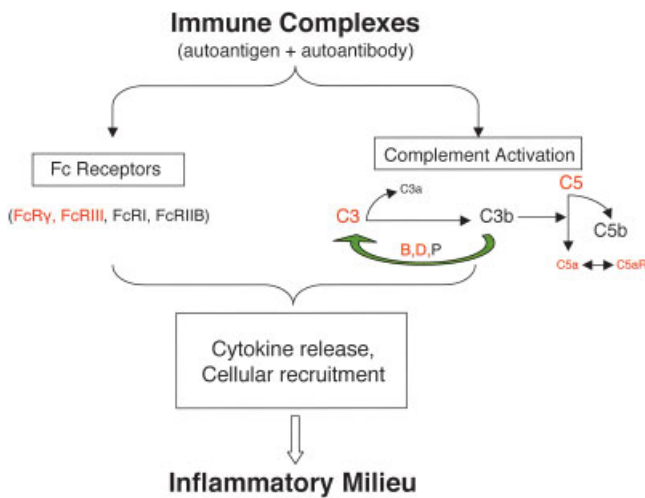
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\* This model needs a new name, because this cumbersome nomenclature is unfortunately indecipherable and meaningless to most rheumatologists. Perhaps, similar to the collagen-induced model of arthritis, it should be called the “GPI-autoimmune model of arthritis.”



**Figure 1.** Early events in the pathophysiology of rheumatoid arthritis. Immune complexes form in the circulation and then deposit in articular tissue or form in situ in the joint. Liberation of anaphylatoxin/chemotactic factor C5a (downstream from C3) and engagement of its receptor are required for arthritis to develop. Mast cell activation, neutrophil recruitment, and cytokine release are necessary to produce synovitis. With disease progression, cytokine release is likely to become a critical factor in orchestrating and perpetuating the disease process. The amplification loop of the alternative complement pathway is shown, because it is required for arthritis to develop in the model. Deficiency of C3, factor B (B), or factor D (D) prevents arthritis from developing. C3b on joint tissue interacts with factor B to form an enzyme complex that cleaves C3 (C3 convertase). Factor D is a serine protease that activates factor B but only upon its binding to C3b. Properdin (P) stabilizes the C3b–factor B enzyme complex. Modification of this enzyme allows it next to cleave C5. Failure to regulate this feedback loop in cartilage is proposed to be the major reason why a joint-centered process develops in the presence of autoantibodies to glucose-6-phosphate isomerase. The receptors and complement components shown in red are required for arthritis to develop in this model.

neous and passive transfer models are informative tools to study the immunopathogenesis of an organ-specific autoimmune disease arising from an adaptive immune response to a self antigen expressed by all cells.

### Requirements for disease in the model

Initiation of arthritis in the K/BxN mouse is attributable to the breakdown of T cell tolerance, with collaboration of autoreactive B cells (3–7). Arthritis does not develop in the  $\mu$ MT mutant mouse, which lacks B cells (6,7). Further studies established that direct interaction of T cells and B cells through CD40/CD40L is necessary for the elaboration of arthritogenic anti-GPI antibodies (4). The subsequent effector phase of arthritis is predominated by cells of innate immunity, macro-

**Table 1.** Similarities between human RA featuring autoantibodies to citrullinated peptides and a mouse model of arthritis featuring autoantibodies to GPI\*

Characteristic	Human RA	K/BxN mouse model
Arises spontaneously	Yes	Yes
Transferred by autoantibodies	Possibly†	Yes
Ubiquitous antigen	Yes	Yes
Joint centered	Yes	Yes
Complement/Fc receptor	Likely	Yes
Mast cell triggering	Likely	Yes
Polymorphonuclear cell recruitment	Likely	Yes
Cytokine release	Yes	Yes

\* RA = rheumatoid arthritis; GPI = glucose-6-phosphate isomerase.

† See ref. 27 for information regarding some of the first attempts to address this issue.

phages, and granulocytes, with a paucity of T cells. Neutrophils are the earliest infiltrating innate immune cells and play a critical role (8–10). Polymorphonuclear (PMN) cell–depleted mice are resistant to the serum-transfer model of arthritis (10). Further, anti-PMN treatment reverses disease progression (10).

Mast cells are detectable in the joint and are activated in human RA. They are required for the GPI-autoimmune model of arthritis (11). Mast cell–deficient mouse strains (W/W<sup>v</sup> or Sl/Sl<sup>d</sup>) are resistant to arthritis induction by serum transfer; mast cell transfer restores susceptibility in the W/W<sup>v</sup> strain (11). Proinflammatory cytokines, particularly interleukin-1 $\beta$  (IL-1 $\beta$ ), tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), and IL-6 are up-regulated in arthritis (6,7). Whereas some TNF $\alpha$ -deficient mouse strains can develop robust disease in the K/BxN mouse serum-transfer model, IL-1<sup>-/-</sup> and IL-1 receptor (IL-1R)<sup>-/-</sup> mice are resistant (11). Intraperitoneal administration of IL-1 $\beta$ , but not TNF $\alpha$ , restores the arthritis susceptibility of W/W<sup>v</sup> mice. This latter result indicates that mast cells contribute to the pathogenesis of arthritis through their production of IL-1 $\beta$  at the site of inflammation.

**Table 2.** Implications for rheumatoid arthritis based on the mouse model

1. Autoantibody-mediated process.
2. The autoantigen is ubiquitous, yet the disease process is joint centered.
3. Rheumatoid arthritis is a syndrome. Future investigators will likely stratify rather than generalize the syndrome of rheumatoid arthritis; that is, based on the patient's autoantibody profile alone, there appear to be distinct diseases within what is currently labeled rheumatoid arthritis, e.g., rheumatoid factor positive versus rheumatoid factor negative, citrullinated peptide positive versus citrullinated peptide negative, and so on.

Antibodies to GPI are detectable in synovial tissue 20 minutes after intraperitoneal injection, and GPI/anti-GPI immune complexes are in the circulation of K/BxN mice (10). Because the pathologic activity of anti-GPI is in the IgG fraction, the role of the Fc receptor (FcR) was examined in the serum-transfer model, using FcR-deficient mouse strains (6). As expected, FcR $\gamma$ -deficient mice are resistant. The high-affinity FcRI plays no role, but the low-affinity FcRIII is important. No inhibitory effect was observed in FcRIIB-deficient mice.

The role of the complement cascade in the K/BxN mouse model of arthritis has also been elegantly described (6,7). By using C5 and C5a receptor-knockout mice, A/J congenic C5-deficient mice, and neutralizing antibodies to C5a, investigators established a critical role for C5a interacting with its receptor. C6 was not required, ruling out participation of the membrane attack complex. As expected, C3, being a gateway to the activation of C5, is essential for the induction of arthritis. Most informative and unexpected was the finding that the classical pathway of complement activation is dispensable, despite the disease being mediated by antibody and featuring immune complex formation. Instead, the alternative pathway is required for the activation of C5. Factor B, but not C4, is necessary, providing additional evidence for the participation of the alternative pathway. In the mouse model, the dominant subclass of the anti-GPI antibodies, IgG1, does not bind C1q and therefore cannot activate the classical pathway. Instead, antibody bound to antigen serves as a site for alternative pathway amplification: the so-called antibody-dependent activation of the alternative pathway (12).

### Local synthesis of complement

In a field pioneered by Harvey Colten and his colleagues, many different cell types have been shown to synthesize complement components (13,14). Although the liver is responsible for supplying plasma with almost all (>90%) of the complement components of the activating cascades (two exceptions being factor D and properdin of the ancient alternative pathway), cells involved in the acute inflammatory reaction synthesize many components. This is especially true of cells of monocyte/macrophage lineage that reside in diverse organs (liver, spleen, lymph node, kidney, peritoneum, lung, skin, brain, breast, and placenta). Resident or elicited peritoneal and alveolar macrophages have been commonly used as a "factory" to assess biosynthesis, especially of the relatively more abundant C1q, C3, C4,

and C5 components. Most of the early knowledge about the biosynthesis of complement components was gained using cells of monocyte/macrophage lineage. Local synthesis of complement components evolved for a purpose, presumably to provide host defense against infection. Local synthesis has been noted in all species examined to date, and has been evaluated extensively in mice, guinea pigs, and humans.

Complement components in body fluids (pleural, pericardial, gastrointestinal, peritoneal, spinal) represent a small percent of plasma concentrations. What part of this is derived from local synthesis versus a filtrate from plasma has not been well delineated. After an inflammatory reaction is under way, >90% of the components (e.g., in a pleural effusion caused by instillation of an irritant or bacteria) are derived from plasma (15). These data do not reveal how the response was initiated. Is local synthesis of components necessary, or is that derived from a filtrate of plasma sufficient?

What, then, is the role of local synthesis? There are two likely possibilities, which are not mutually exclusive, and evidence exists to support both. One possible role of local synthesis is to initiate the local inflammatory response, and the other is to facilitate the innate/adaptive immune response. A simple paradigm relative to the first possibility is that resting/resident monocyte/macrophages serve as a factory to secrete components (13–15). They maintain a sufficient level of complement-activating potential to alert the local environment to injury and infection. After an inflammatory response has been initiated, further complement activation via local components is no longer necessary. The local response team has now been augmented many-fold by the influx of plasma. For example, murine peritoneal macrophages placed in culture secreted a decreasing quantity of C4 over the first 24 hours (16,17). At the same time, they were becoming progressively more activated. Macrophages elicited with agents such as thioglycolate secrete minimal C4 in culture but are highly phagocytic. In sum, resident phagocytic cells appear to change their activity profile from that of a secretory cell-producing reagent to initiate an inflammatory response to that of a cell now primed to phagocytose and kill microorganisms. This is a logical and desirable sequence of events in response to an infectious challenge.

Complement component C3 is an abundant plasma protein (1–2 mg/ml). Hepatocytes synthesize a single-chain C3 precursor that is cleaved intracellularly to a two-chain, disulfide-linked mature form that is then secreted into the blood. C3 is an acute-phase protein,

the level of which increases up to two-fold in inflammatory conditions. More than 90% of circulating C3 is contributed by the liver. C3 biosynthesis has been studied in a wide range of human cells, monocyte/macrophages, T cells, and B cells, but also in endothelial cells and alveolar and renal tubular epithelial cells. Neutrophils, which play a key role in the initiation of arthritis in the K/BxN mouse model (10), constitutively synthesize low amounts of C3 but have the capacity to increase production in response to stimuli. In vitro stimulation of neutrophils with TNF $\alpha$  increased the production of both C3 messenger RNA (~50-fold) and protein (~6-fold) (18).

The availability of C3<sup>-/-</sup> mice has permitted performance of a series of interesting experiments to examine the role for extrahepatic C3 synthesis in immune responses (19–21). Bone marrow–derived cells provide sufficient local C3 synthesis for priming an adaptive antibody response. Evidence supporting this hypothesis has come from the bone marrow chimera studies of C3<sup>-/-</sup> mice (19–21). Radiation is used to eliminate the sensitive bone marrow cells in C3<sup>-/-</sup> mice. Bone marrow from WT mice, containing cells secreting C3, is transferred to create chimeric mice in which the level of circulating C3 is negligible. C3 synthesized by WT mouse bone marrow–derived cells restores the antibody response against intradermally administered herpes simplex virus (HSV) (21). In the reverse situation, in which C3<sup>-/-</sup> bone marrow reconstituted WT hosts, the chimeric mice failed to generate a sufficient B cell response to cutaneous HSV infection (even with a normal C3 level in the circulation). Interestingly, if HSV was administered intravenously, the deficient antibody response in the reverse chimeric (C3<sup>-/-</sup> bone marrow into an irradiated WT host) was normalized. The antibody response profiles from chimeric mice paralleled the size of germinal centers. Similar results were obtained with C4<sup>-/-</sup> mice. Together, these experimental data support the hypothesis that locally synthesized complement components, at least in the skin, play a critical role in initiating a robust adaptive antibody response.

#### No requirement for local synthesis of C3 in this mouse model

In a study reported in this issue of *Arthritis & Rheumatism*, Monach et al (22) used a clever experimental design to address the question of the role of local synthesis of complement components in the K/BxN-TCR mouse model of RA. Those investigators used the

**Table 3.** Key questions about the immunology of rheumatoid arthritis

1. How is tolerance to ubiquitous self antigens broken?
2. Why is the process joint centered?
3. What perpetuates the immune response?

serum (antibody)–transfer model in the setting of C3<sup>-/-</sup> mice, bone marrow chimeras, and parabiotic animals. They conclusively demonstrated that local synthesis by monocyte/macrophages of complement components is *not* required to induce arthritis, i.e., the arthritis phenotype is not influenced by a lack of local synthesis of complement components.

A few caveats must be considered. As noted, Monach and colleagues performed their analysis in a transfer system in which pathogenic IgG antibodies reacting with GPI were injected into recipients. The primary function of local synthesis of complement may be to alert the host to the presence of an infectious agent. Injecting preformed antibodies to initiate joint inflammation is not likely to closely mimic HSV infection of the skin, which is the one situation in which local synthesis has been shown to facilitate an adaptive immune response to this pathogen (19–21). Thus, these data do not eliminate a role for local synthesis of complement components in facilitating a *de novo* immune response. Further, they do not tell us what would be the outcome at other tissue sites and in body cavities and fluids.

These data are reminiscent of those involving C4-deficient guinea pigs, from several decades ago (23). This time, the model under investigation was the Forssman shock model. Guinea pigs are Forssman antigen (a lipopolysaccharide antigen) positive, while rabbits are Forssman antigen negative. Therefore, antibodies to the Forssman antigen can be raised in rabbits. Upon intravenous injection of such antibodies into guinea pigs, they travel to the lung, bind the Forssman antigen to form immune complexes, and then activate the classical complement pathway. Pulmonary edema, leading to hypoxic death, occurs in a few minutes. C4-deficient guinea pigs are resistant to Forssman shock. Upon reconstitution, however, with just a small percentage of the normal concentration of plasma C4, they now behave similarly to C4-sufficient animals. Interestingly, if the Forssman antibodies are injected during the initial 12 hours after C4 reconstitution, the animals do not die. If one waits until 24 hours to inject the anti-Forssman antibodies, death occurs. The interpretation of these data is that it takes a few hours for the C4 introduced into plasma to

distribute to the interstitial space. A second lesson, analogous to what was reported by Monach et al, is that local synthesis is not required. Instead, sufficient complement components accumulate in the interstitial space, derived from plasma, to trigger the complement system.

The data in this report are an example of how the K/BxN mouse serum-transfer model can be used to explore questions of importance for rheumatologists trying to understand how autoantibodies contribute to the pathogenesis of RA (Table 2). Although an initial report claimed a high percentage of anti-GPI antibodies among patients with RA, this result was not confirmed in subsequent studies of patients with RA or those with juvenile RA (24–26). At this stage, the consensus is that antibodies to GPI are uncommonly found and are not specific for human RA. Nevertheless, they provide a mechanism for how autoantibodies such as those to anti-citrullinated peptide antibodies might cause RA (27).

### Conclusion

Along this line of reasoning, we conclude by pointing out parallels between RA featuring IgG antibodies to citrullinated peptides and IgG antibodies to GPI. First, citrullinated peptides are produced in all organ systems. GPI is a ubiquitous enzyme. Second, despite this, in both cases the disease process is joint centered. Third, the autoantibodies are of the IgG class, and the disease is transferable with serum or with purified IgG. Fourth, how and why these antibodies arise are unclear. Fifth, the concept of scarcity (or absence) of complement regulatory proteins in cartilage is a prominent aspect of the discussion of disease pathogenesis in reports on the mouse model (6). In other words, the antibodies bind to many tissue sites, but the inflammatory reaction is “successful” only in triggering disease in a certain anatomic site—in this case, the joint. In the mouse model of RA, a peculiarity of the host’s distribution of regulators of complement has been proposed (6,7). It is our opinion that further analysis of this and related models, particularly in relation to the role of anti-citrullinated peptide antibodies in human RA, will be fruitful (Table 3). Hopefully, such investigations will continue to improve our understanding of the immunopathogenesis of RA.

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