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Comments
At the time of publication, Ellen Gravallese was not yet affiliated with the University of Massachusetts Medical School.

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Critical Roles for Interleukin 1 and Tumor Necrosis Factor in Antibody-induced Arthritis

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Abstract

In spontaneous inflammatory arthritis of K/BxN T cell receptor transgenic mice, the effector phase of the disease is provoked by binding of immunoglobulins (Igs) to joint surfaces. Inflammatory cytokines are known to be involved in human inflammatory arthritis, in particular rheumatoid arthritis, although, overall, the pathogenetic mechanisms of the human affliction remain unclear. To explore the analogy between the K/BxN model and human patients, we assessed the role and relative importance of inflammatory cytokines in K/BxN joint inflammation by transferring arthritogenic serum into a panel of genetically deficient recipients. Interleukin (IL)-1 proved absolutely necessary. Tumor necrosis factor (TNF) was also required, although seemingly less critically than IL-1, because a proportion of TNF-deficient mice developed robust disease. There was no evidence for an important role for TNF for bone destruction. The variability in the requirement for TNF, reminiscent of that observed in treated rheumatoid arthritis patients, did not appear genetically programmed but related instead to subtle environmental changes.

Key words: transgenic • cytokine • knockout • inflammatory • TNF

Introduction

Inflammatory arthritides, in particular rheumatoid arthritis, have been the focus of intense investigation, but their etiology and pathogenesis remain controversial. There is no consensus on what initiates rheumatoid arthritis (RA)*; i.e., whether it is primarily an autoimmune response, an inflammatory response to some persistent microbial invasion, or a combination of the two. There is also dispute over the leukocyte populations that are involved in the initiation of joint inflammation. The paradigm currently dominating the field portrays antigen-specific T cells in the joint as inducing the inflammatory cascade by triggering macrophages and synoviocytes (1, 2), but this scenario has been questioned for a lack of direct experimental demonstration of certain of its key points, and because of some discordant observations, such as the paucity of T cell-derived cytokines in inflamed joints (3). In contrast, a role for inflammatory cytokines like TNF and IL-1 is well established (4), but these are demographically by the in vivo effect of therapeutic protocols that block TNF-TNF-R interactions (1). There has also been debate on the relative importance of the IL-1 and TNF pathways (4). It has also been noted that, even in the best of trial outcomes, arthritis is not fully reversed and roughly one third of RA patients are refractory to TNF-F-R-blocking drugs.
RNA Analysis. RNA was prepared from ankle tissue by a modification of the LiCl/urea technique (22), designed to avoid contamination of the joint RNA with bone marrow-derived material by leaving the bone intact. A fine dissection of ankles (sectioned at the long bones of the lower leg and in the metatarsal area), the tissue was freed of skin and superficial tendons. The joint was immersed in 1 m LiCl solubilization solution (6 M LiCl, 2% SDS). AArticular cavities were opened with a scalpel and were exposed to the medium to release the cellular contents. After 10 m incubation, fragments were removed, and an equal volume of concentrated LiCl isolation (6 M LiCl, 6% Urea, and 10 mM sodium acetate, pH 5) was added to precipitate the RNA. cDNA was synthesized from these RNAs by MuLV reverse transcriptase (GIBCO BRL).

Cyclophilin was used as an endogenous control using a probe concentration of 200 and 400 nM for each primer in each reaction. The probe and primer sequences used are as follows: probe, 5'-CTGCTGGCTGAGGTGTTG-CCACATCCTGAA-3'; and reverse primer, 5'-TGCTTTTGAACCTTGTTGCTCGAA-3'. For the quantification of TNF and IL-6, the TaqMan predeveloped assay reagents were used (PE, Applied Biosystems). For IL-1, the probe and primer concentrations per reaction were the same as those used for cyclophilin. The probe and primer sequences used are as follows: probe, 5'-FAM-CTGACGCTGAAGTGTTGCCATCCATTA-3'; and reverse primer, 5'-TGAAGAGC-GCACAGGAC-3'.

Serum Transfer Protocol and Arthritis Scoring. K/BxN serum pools were prepared from arthritic mice 60 d old. Arthritis was induced by intraperitoneal injection of 150–200 ul serum into each hind paw of 3 mice. For each animal, paw and arthritis were evaluated on a 0–4 scale (0, no redness or only a few digits affected; 4, both paws red and swollen). An arthritis index was calculated for each animal: arthritis index = (paw + arthritis) x 2. A clinical index was evaluated over time for each animal (0 point for each affected paw; 4 points for a paw with only mild swelling/redness or only a few digits affected). Arthritis thickness was measured by a caliper (6), with ankle thickness being defined as the difference in ankle thickness from the day 0 measure. Histology. Hind limb were collected and the knee and ankle joints were separated and fixed in 10% formal saline solution for 24 h.

Materials and Methods

Mice. The knock out mice used for serum transfer were obtained from the Jackson Laboratory, housed in our animal facility at Harvard Medical School animal facility at 4–5 wk of age, and used 1–3 wk later (in rare exceptions, the mice were housed in our colony). These mice include the following: IL-6 (5); IL-1β (5); TNF-α (5); IFN-γ (5); IL-12 (5); IFN-β (5); IL-10 (5); and IL-4 (5).

Histology. Hind limb were collected and the knee and ankle joints were separated and fixed in 10% formal saline solution for 24 h. Specimens were dissected to remove skin and outer muscle, and subsequently fixed in 4% paraformaldehyde for 1 h. The specimens were then rinsed in PBS and embedded in paraffin (Paraplast, 1000; Shandon). For each specimen, twenty 4-μm sagittal serial sections were cut, 10 per section, and every fifth section was stained with hematoxylin and eosin (1 μg/mL). Specimens were cut, 10 per section, and every fifth section was stained with hematoxylin and eosin (1 μg/mL) for specific identification of proteoglycan. Histopathological scoring was performed as described previously (6, 23).

Results and Discussion

Kinetics of Inflammatory Cytokine Production. Transfer of K/BxN serum into normal recipients induces rapid and synchronous development of arthritis, the first signs of joint inflammation appearing within 24 h in fully susceptible strains (9). To begin exploring the induction of various inflammatory cytokines in this model, we used quantitative real-time PCR. C57BL/6 mice were infected with the K/BxN TCR transgenic mouse is a recently developed model of inflammatory arthritis (5–9). A ILK/BxN animals spontaneously show an autoimmune disease with m ost (although not all) of the clinical, histological, and immunological features of RA in human. The disorder is critically dependent on both T and B cells. Although the pathologic manifestations are joint-specific, the process is initiated, and then perpetuated, by dual T/B cell autoactivity to a ubiquitously expressed antigen, glucose-6-phosphate isomerase (GPI). Transfer of anti-GPI IgGs from arthritic K/BxN mice into healthy mice also brings autoactivity within days, even when the recipients are devoid of lymphocytes. GPI-anti-GPI immune complexes (ICs) are the link between the system in T and B lymphocyte autoactivity and the ensuing joint-specific inflammatory and destructive; the joint specificity is perhaps a reflection of the presence of GPI on the articular cavity surface (10). 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 contains anti-GPI Abs, absent from serum of normal individuals or of patients with lupus nephritis or Sjogren’s syndrome (12), although in one recent data show less obvious a correlation (unpublished data). The observation of GPI and GPI-anti-GPI complexes on cartilage surfaces of human joints is also of interest (10).

Our early studies on K/BxN mice revealed augmented local synthesis of inflammatory cytokines, such as IL-6 and TNF-α, in arthritic joints (5). However, the functional relevance of this observation was not tested, other than a report that failed to demonstrate a required role for TNF-α (13). The role of inflammatory cytokines in an immune disease in the joint is independent of the variant sequence of human joints or rather to the variants resistant to TNF-induced arthritis, bone erosion, and cartilage destruction. An adjacent serial section was performed as described previously (6, 23).
mice lacking particular cytokines or cytokine receptors. Mice of matched genetic composition, bred in the same colony, were used as controls. In most cases, we preferred not to rely on injected cytokine inhibitors, such as anticytokine antibodies or soluble receptor molecules because negative results with such reagents can be difficult to interpret (sufficient dose or stability of the compound? completeness of the blockade?). This is particularly an issue in a context as aggressive as that of K/BxN arthritis.

We first investigated the importance of IL-6, a pleiotrophic cytokine expressed by a variety of cell types during inflammatory processes (24). IL-6 has complex pro- and antiinflammatory effects, with both local and systemic effects. For example, in primate sera in uine responses and plan a cell and macrophage differentiation (25), but also induces acute phase proteins. IL-1 receptor antagonist (26), and metalloproteinase inhibitors (27). Its role is variable in different inflammatory models (28). These have been conflicting reports of the requirement for IL-6 in animal models of arthritis, some investigators describe reduced disease in IL-6-deficient mice or after antibody blockade of its receptor (29, 30), whereas others report no such effect (31).

IL-6-deficient mice on the C57Bl/6 background (14) were transfected with serum from arthritic K/BxN mice, and arthritis development was compared as described previously (6). The representative experiment in Fig. 2A demonstrates a very similar arthritis course in IL-6-deficient and control mice. The initial onset of symptoms was as the same, all distal joints were affected, and with a comparably degree of inflammation (measured as ankle thickness). These observations were confirmed by results from three individual experiments tabulated in Fig. 2B. Histological examination of the ankle joints revealed the same degree of synovitis and joint infiltration typical of K/BxN mice (synovial thickening and infiltration, presence of neutrophils in the articular cavity, pannus formation, and cartilage destruction; Fig. 2 C; unpublished data). Furthermore, cartilage and proteoglycan loss was evident in toluidine blue-stained ankle sections from serum-injected mice at comparable lesion levels for IL-6-deficient and control mice (unpublished data).

These data are in agreement with those of van den Berg and colleagues, who found little role for IL-6 in joint inflammation in CIA or zymosan-induced arthritis (31). They contrast with other reports showing an effect of IL-6 blockade in the CIA model (29, 30). The explanation for these discrepancies may lie in the positive impact of IL-6 on the immunological initiation phase of the CIA model: less intense immune responses were made to the collagen-II antigen in the absence of IL-6 function (29, 30). Together, these data are consistent with the notion that IL-6 does not play a major role in the inflammatory effector phase of arthritis.

An Essential Role for IL-1. Although attempts at blocking the IL-1 pathway in RA patients in therapeutic trials have not met with as much success as those interfering with the activity of TNF, there exists a substantial body of evidence in complicating this inflammatory cytokine in several
classic murine arthritis models, whether autoimmune in nature or induced by local microbial particles (32–36); similarly, high levels of IL-1 transcripts have been detected in RA synovium (4, 37).

We tested the susceptibility to serum-transferred arthritis of the IL-1R knockout strain (15), in which neither IL-1 nor IL-1R-mediated signals are possible. After K/BxN serum transfer, essentially no clinical signs of disease were observed in the IL-1R-deficient mice, except for a limited swelling of the digits and a slight flattening in the ankle-thickness curve (Fig. 3). To guard against possible influences of genetic background variability, we repeated the initial experiments performed in B6 (129)F2 mice in IL-1R–deficient and control mice (matched for gender/age and genetic background) (our standard fully susceptible background; reference 11). Matched wild-type controls responded as expected from a representative experiment, with each curve representing an individual mouse. B) Tabulation of the results for 10 knockout mice and age/gender-matched controls on either the standard B6 (129)F2 background or on an inbred B6 background. Scoring was described as for Fig. 2; the star denotes a transient inflammation in the digits of one mouse.

These clear-cut results indicate that, in this serum-transferred arthritis model, IL-1 plays a central and indispensable role (for review see 1). We also investigated the effect of knockout mutations of the genes encoding TNF-α or its close homologue, lymphotoxin (LT)–α (17–21). TNF-α and LT–α mediate their pleiotropic effects by binding to one of two known receptors; TNF FR 1 (p55) and TNF FR 2 (p75).

Inflammation Cytokines in K/BxN Arthritis

Figure 2. No requirement for IL-6 in arthritis induced by K/BxN serum transfer. IL-6-deficient and control mice (mated for gender/age and genetic background) were injected with 150 μl serum from arthritic K/BxN donors on days 0 and 2. A) A threshold was evaluated by means of clinical index and ankle thickness (M and N methods). B) Data from a representative experiment, with each curve representing an individual mouse. B) Tabulation of the results for 10 knockout mice and age/gender-matched controls on either the standard B6 (129)F2 background or on an inbred B6 background. Scoring was described as for Fig. 2; the star denotes a transient inflammation in the digits of one mouse.

Figure 3. Essential role of IL-1. IL-1R–deficient and control mice (mated for gender/age and genetic background) were injected with 150 μl serum from arthritic K/BxN donors on days 0 and 2. Arthritis was evaluated by means of clinical index and ankle thickness as in Fig. 2. A) Data from a representative experiment, with each curve representing an individual mouse. B) Tabulation of the results for eight knockout mice and age/gender-matched controls on either the standard B6 (129)F2 background or on an inbred B6 background. Scoring was described as for Fig. 2; the star denotes a transient inflammation in the digits of one mouse.

These results evoked models of arthritogenesis in which TNF-α plays a central and indispensable role (for review see 1). We tested the efficacy of K/BxN serum transfer in animals carrying knockout mutations of the genes encoding TNF-α or its close homologue, lymphotoxin (LT)–α (17–21). TNF-α and LT–α mediate their pleiotropic effects by binding to one of two known receptors; TNF FR 1 (p55) and TNF FR 2 (p75).

We also investigated the effect of knockout mutations of the genes encoding either or both of these molecules. The data, summarized in Table I, allow several conclusions. First, and most importantly, IL-1 plays a central role, critically required for disease progression. We have not been able to reproduce this effect by treatment with blocking anti–IL-1R mAb (unpublished data), likely because of the known difficulty to achieve complete blockade of IL-1 action with biologic inhibitors (for review see reference 4). The central role of IL-1 in the K/BxN model is reminiscent of its requirement in CIA and other murine arthritis models (32, 33, 35). It is also consistent with the finding that intracellular expression of IL-1α, alone, is sufficient to induce full-blown arthritis (38).

TNF Family Influences. Members of the TNF family have received a great deal of attention in the context of inflammatory arthritis. This has ranged from the initial demonstration of TNF-α expression in synovium, to establishing the efficacy of TNF–α/TNFFR1–/TNFR2– blocking agents in animal models, to the successes of such reagents in therapeutic intervention in human RA (1, 4, 39–42). Aberrant expression of TNF-α is also sufficient to induce arthritis in transgenic animals (43). These results evoked models of arthritogenesis in which TNF-α plays a central and indispensable role (for review see 1). We tested the efficacy of K/BxN serum transfer in animals carrying knockout mutations of the genes encoding TNF-α or its close homologue, lymphotoxin (LT)–α (17–21). TNF-α and LT–α mediate their pleiotropic effects by binding to one of two known receptors; TNF FR 1 (p55) and TNF FR 2 (p75).

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23 examined over the course of this study. This finding is illustrated for representative cohorts in Fig. 4. The presence of responder TNF\(^{-}\) mice was not restricted to one or two experimental groups, but was observed in a number of independent experiments. In contrast, a certain degree of clustering was observed, some experimental groups showing a high incidence of arthritis development (see below). When disease did develop, the time of onset was quite variable, usually delayed by several days relative to wild-type controls, and the degree of inflammation always remained below the maximum attainable. Histological analysis also revealed significant signs of inflammation in those mice with clinically detectable arthritis.

Third, joint inflammation developed normally in both the TNFR1- and TNFR2-deficient mice, as well as in TNFR1/TNFR2 double–deficient animals (Table I; the genotypes of the mice were reconfirmed at the end of the experiment). Clinical and histological parameters were essentially indistinguishable from normal controls. This observation was quite unexpected, as TNFR1 and TNFR2 are the only known receptors for TNF\(^{-}\), with no reported indication of another possible receptor in spite of the broad attention that TNF\(^{-}\) has received (44). As both the cytokine and cytokine receptor mutations were on a susceptible (B6 \(\times\) 129F2) background, one would have expected that they have the same phenotype in both deficient strains.

These conflicting results prompted us to question the effect of the TNF\(^{-}\) mutation: was the poor responsiveness in TNF\(^{-}\)-deficient mice truly due to the absence of the cytokine, or instead to some independent factor (a linked gene effect, quite plausible given the genomic localization of the TNF\(^{-}\) locus; an independent mutation; protective genes segregating by chance, etc.)? If the former were true, it should be possible to complement the deficiency by TNF\(^{-}\) replacement, e.g., by triggering TNF\(^{-}\)R1 with an agonistic mAb. To test this prediction, we injected cohorts of TNF\(^{-}\)-deficient mice with K/BxN serum, selected those mice that remained free of arthritis after 7 d, and administered the agonistic anti-TNFR1 Ab 55R-593 (45). As shown in Fig. 5, the Ab had a marked effect, provoking arthritis in all the TNF\(^{-}\)-deficient mice that had previously received K/BxN serum. No arthritis was observed when 55R-593 was injected without serum pretreatment (unpublished data). Several control Abs were used in parallel to rule out trivial explanations for this observation: an isotype-matched control Ab, anti-TNFR1 Abs with blocking or antagonist activity (55R-170, 55R-286). None of these reagents induced arthritis (Fig. 5 B), at least not beyond the minority of TNF\(^{-}\)-deficient mice one might expect to eventually progress spontaneously to arthritis on the basis of the results presented in Fig. 4. Thus, results from these experiments confirm that TNF\(^{-}\)-deficient mice indeed lack the element missing in TNF\(^{-}\)-deficient mice that is required for robust development of arthritis.

### Table I. Arthritis Incidence in Mice Deficient in TNF and TNFR Families

<table>
<thead>
<tr>
<th>Strain</th>
<th>Arthritis</th>
<th>Days of onset</th>
<th>Max CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFR1/8/8 (B6)</td>
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<td>3,4,4,4,4,4,3</td>
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<tr>
<td>TNFR2/8/8 (B6)</td>
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<tr>
<td>TNFR1/2/6/6x129F2</td>
<td>2,1,4,2,2</td>
<td>4,4,4,15,35,3</td>
<td></td>
</tr>
<tr>
<td>Lt/8/8x129F2</td>
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<td>4,4,4,4,4,4,3</td>
<td></td>
</tr>
<tr>
<td>TNF/9/9x129F2</td>
<td>2,2,3,5,4,2,3,3</td>
<td>25,4,4,2,4,2,5,35</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4. Variability of arthritis in TNF\(^{-}\)-deficient mice. TNF\(^{-}\)-deficient (left) and control mice (right; matched for gender/age and genetic background) were injected with 150 μl serum from arthritic K/BxN animals on days 0 and 2. Arthritis was evaluated by measuring ankle thickening as in Fig. 2. The data are pooled from six different experiments. All mAbs originated from the Jackson Laboratory.
Further experiments were performed to address the cause of the variable effect of the TNF- deficiency. It could be explained by genetic, epigenetic, or environmental variation controlling the activity of TNF- -independent pathways; stochastic threshold effects could also be involved, arthritogenesis resulting from a certain degree of local inflammatory insult, only seldom reached in the absence of TNF- . As the knockout mutation was carried on a mouse strain (129x16) F2 background, we reasoned that modifier alleles at other loci, able to complement the TNF deficiency, might segregate randomly in the F2 knockout mice. To test this hypothesis, several crosses were set up between combinations of resistant or susceptible TNF- -deficient mouse strains. Should alleles at independent loci be segregating, there should be heritable transmission of these traits to the progeny. As shown in Fig. 6 A, this was not the case. A cross between two resistant strains yielded a dominant proportion of responder mice; the transgenic mice of a recessive susceptibility allele in this family would be very unlikely to yield such a pattern (P  0.001). Thus, the variability does not stem from Mendelian genetic elements. Epigenetic variation, however, could perhaps account for these results. How ever, we observed a clear correlation between the origin and life history of the mice and their responses to K/BxN serum (Fig. 6 B). Mice bred at the Jackson Laboratory and shipped to Boston 7–15 d before challenge showed mainly a resistant phenotype, whereas those bred in Boston and tested there were mainly susceptible (P  0.003). In both cases, the barrier facilities have SPF status, free of major mouse pathogens, but minor bacterial flora varies. Thus, the segregation of responses is consistent with an environmental explanation rather than with an epigenetic one.

Together, these experiments point to a distinct involvement of TNF- in Ab-induced arthritis, but one that is not absolutely essential. This conclusion differs from that reached by Kyburz et al. (13), who found no effect of anti-TNF- therapy in arthritis development in straight K/BxN transgenic mice. We have also made similar observations, injecting several different anti-TNF- reagents into young K/BxN mice (unpublished data). However, we interpret these negative results with caution because of the very aggressive nature of the disease that develops in the transgenic mice and uncertainties concerning the efficiency of Ab-meditated blockade. On the other hand, the present results do concur with reports of indolent development of CIA in TNF- -deficient mice (46). Although it is conceivable that the cytokine network adaptates somewhat in TNF- -deficient animals, it is much less likely that such adaptational effects are at play, the degree of TNF- involvement being dependent on the general inflammatory state of the individual. It should be worthwhile trying to pinpoint what these influences might be, in both mouse and human models, and the present system provides a handle.

There are several potential interpretations for the strong arthritis that develops in TNF- -deficient mice. The most straightforward is that other receptors can compensate for an absence of TNF- signals. Although the existence of such a receptor has not been reported to date, the presence of the TNF family is quite possible that other receptors will be found to bind TNF- . Whether these are indeed the primary receptors mediating arthritis, or whether they are secondary, or at play when the primary TNF receptors are absent, will need to be explored. Alternatively, one might propose that TNF- -independent arthritis pathways are particularly active when TNF FR 1 is missing, perhaps by com mediating downstream signal transduction adapt-
tors. For example, the absence of TNFR1 might free TRADD, FADD, or TRAF molecules for more efficient interaction with other receptors.

Bone Destruction and Formation. There is some debate about the role of inflammatory cytokines in promoting focal bone erosion in the course of arthritic diseases. Osteoclasts are essential to the process, and essentially no focal destruction of the bone occurs in their absence. Resistance to bone erosion was previously demonstrated in mice deficient in the TNF family member receptor activator of NF-κB ligand (RANKL) that had received K/BxN serum, as in the CIA model after blockade of RANKL by osteoprotegerin treatment (23, 47). This finding is consistent with the fact that RANK/RANKL axis is required for the generation of osteoclasts and also plays a role in their activation (for review see reference 48). In contrast, it is also possible that other inflammatory cytokines play a role. IL-1 can activate osteoclasts, and promote bone resorption in vitro (49, 50). TNF promotes osteoclast differentiation in the presence of RANKL (51, 52), and there are indications that TNF/TNF receptor blockade can retard bone destruction in RA patients, even when the effect on the inflammatory component is limited (53). Thus, we asked whether bone destruction could be seen in the absence of these cytokines.

As described previously, obvious instances of focal bone destruction were seen in normal mice injected with K/BxN serum; similar images were also observed in LT-deficient mice (Fig. 7, A and B). For TNF−/−, we focused in particular on those mice that showed significant joint inflammation. In these instances, clear evidence of focal bone destruction was also observed (Fig. 7 C). Although impossible to truly quantify, given the variability of inflammation in the TNF-deficient animals, the extent of the erosive lesions in the absence of TNF−/− was largely on par with the extent of inflammatory joint disease.

We could not draw any conclusion on the role of IL-1 in bone destruction, as the upstream inflammatory phase did not develop in its absence. However, our results are not consistent with the view that TNF−/− plays an obligate role in promoting bone destruction; synovitis and joint inflammation could still lead to extensive destruction in its absence.

Synthesis: Intersection of IL-1 and TNF Pathways. There has been quite some debate as to the relative roles and importance of IL-1 and TNF−/− in arthritogenesis. In animal models where the function of these cytokines has been tested, their in postpartum effect varies according to the disease-eliciting agent, although IL-1 may play an important role in the cartilage and bone destruction that ultimately ensues (for review see reference 4). For Ab-mediated arthritis that the K/BxN disease may typify, our results point to a more crucial function for IL-1. These roles, and the slightly different kinetics of induction of cytokine transcription in the joint during arthritis initiation, are consistent with a model in which the point of action of TNF would be upstream of that of IL-1 (1). TNF−/− independent pathways, perhaps relying on other members of the TNF family, may also trigger IL-1 independently. This view is consistent with the importance of TNF−/− in promoting IL-1 production by synovocytes in RA patients (54), or with the fact that IL-1 blockade prevents the arthritis induced by transgene-encoded TNF−/− expression (54). It should also be pointed out that the experiments shown in Fig. 1 only detect transcriptionally induced TNF−/− production. However, it is likely that even earlier release of TNF−/− occurs in the first minutes or hours of the disease, released from intracellular stores of synovocytes or mast cells upon triggering by C5a or FcγRIII. These molecules constitute two essential links between the anti-GPI Abs and the inflammatory manifestations of K/BxN arthritis (11), and both pathways are known to precipitate rapid TNF−/− release.

The relevance of the Ab-mediated arthritis model that K/BxN mice present to human arthritic diseases had been questioned, in part, because it does not fit well with the paradigm in which autoreactive T cells within the joint provoke local TNF−/− release, a model bolstered by the
successes of anti-TNF therapy. The present results show that arthritis induced by Ab complexes in the joint also end up with the production of TNF-α and IL-1, and is strongly dependent on these cytokines.

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