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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 IL-6. Bone destruction and reconstruction were also examined. We found that all mice with strong inflammation exhibited the bone erosion and reconstruction phenomena typical of K/BxN arthritis, with no evidence of any particular requirement 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.

Keywords: transgenic • cytokine • knockout • inflammatory • TNF

Introduction

Inflammatory arthritis, 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 persisting microbial invasion, or a combination of the two. There is also a 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 initiating 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), most demonstratively by the impressive 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–TNFR-blocking drugs.
The K/BxN TCR transgenic mouse is a recently developed model of inflammatory arthritis (5–9). A ILK/BxN animal spontaneously shows an autoimmune disease with m costs (although not all of the clinical, histological, and immunological features of RA in humans. 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 IgG from arthritic K/BxN mice into healthy animals provokes arthritis within days, even when the recipients are devoid of lymphocytes. GPI-anti-GPI immune complexes (ICs) are the link between the system T and B lymphocyte autoactivity and the ensuing joint-specific inflammatory ation and destruction; the joint specificity is perhaps a reflection of the presence of GPI on the articular cavity surface (10). Initiation of the inflammatory effector phase requires both the IFN- 

The K/BxN model of RA is supported by a recent report that serum from all but two thirds of RA patients contains anti-GPI Abs, absent from serum of normal individuals or of patients with lymphoid malignancies or Sjogren’s syndrome (12), although a recent study show less obvious a correlation (unpublished data). The observation of GPI and anti-GPI complexes on cartilage surface 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 phasic relevance of this observation was not tested, other than a report that failed to dem onstrate a required role for TNF-α (13). The role of inflam matory cytokines in an in vitro assay is considered in an attempt to relate the mechanistically defined mouse model to human RA patients. For exam ple, does Ig-induced arthritis correspond to the TNF-α-dependent form of the human disease rather than to the variance resistant to TNF-α blockade?

Here, we apply the K/BxN serum transfer system to a panel of mice deficient 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 in vitro assay is considered in an attempt to relate the mechanistically defined mouse model to human RA patients. For example, does Ig-induced arthritis correspond to the TNF-α-dependent form of the human disease rather than to the variance resistant to TNF-α blockade?

Results and Discussion

Kinetics of Inflammatory Cytokine Production. Transfer of K/BxN serum into normal recipients induced rapid and synchronous development of arthritis in all mice. The onset of arthritis appeared within 24 h in fully susceptible strains (9). To begin exploring the induction of various inflammatory cytokines in this model and their temporal relationships, we measured the expression of their mRNAs by quantitative real-time PCR. C57Bl/6 mice were infected with the LCR/1 virus technique (22), designed to avoid contamination of the joint RNA with bone marrow-derived material by leaving the bone intact. A single section of ankles (sectioned at the long bones of the lower leg and in the metatarsal area), the tissue was fixed of skin and superficial tendons. The joint was immersed in 1 mL of RNA isolation solution (6 mL, 2% SDS). A total of ankles were washed with a wash and were exposed to the medium to release the cellular content. After 10 min of incubation, the joint was removed, and an equal volume of concentrated LiCl solution (6 mL, 16 mL, and 10 mM sodium acetate, pH 5) was added to precipitate the RNA. DNA was synthesized from these RNA by M unethical reverse transcriptase (GIBCO BRL).

Cyclophilin was used as an endogenous control using a probe concentration of 200 and 400 mM for each primer in each reaction. The probe and primer sequences were as follows: probe, 5’-VIC CTTGGGCGCGCTCTTT TAMRA 3’; forward primer, 5’- CAGACGCCATGCTGTCTTT-3’; and reverse primer, 5’- TGTCTTTGGAACTTTGTCTGCAA 3’. For the quantification of TNF-α and IL-6, the Tagman premade assay reagents were used (PE, Applied Biosystems). For IL-1β, the probe and primer concentrations per reaction were the same as those used for cyclophilin. The probe and primer sequences were as follows: probe, 5’-FAM TCGAGCTGGAGAGCTTGATGAGCCCA TAMRA 3’; forward primer, 5’-TGAAACGGCAGCACCCACCA 3’; and reverse primer, 5’-AACCGCTTTTCGACCTCTCTCTT 3’. To determine relative expression values, CΔCt (cyclophilin; CΔT cytokine) was used to derive an expression index (2−ΔΔCt), which was then divided by the value of a reference sample of total spleen RNA.

Serum Transfer Protocol and Arthritis Scoring. K/BxN serum pools were prepared from arthritic mice 60 days old. A arthritis was induced by intraperitoneal injection of 150–200 μL serum at days 0 and 2. A clinical index was evaluated over the first 4 wk of observation. An arthritis score was assigned by a caliper (6), with joint thickness being defined as the difference in ankle thickness from the same ankle in mice not treated with arthritis.

Histology. Histological sections were stained with toluidine blue. Specimens were collected and the knee and ankle joints were separated into right and left. Specimens were dissected to remove skin and outer muscle, and subsequently fixed in 4% formaldehyde for a minimum of 12 h and then in 70% ethanol for 3 days. For each specimen, twenty four-sagittal serial sections were cut, and every fifth section was stained with hematoxylin and eosin (SE) and with toluidine blue (SE-Antibodies) for specific evaluation of proteoglycan. Histopathological scoring was performed as described previously (6,23).

MATERIALS AND METHODS

Mice. The knockout mice used for serum transfer were obtained from the Jackson Laboratory, brought to our animal facility at the Harvard Medical School animal facility at 4–5 wk of age, and used 1–3 wk later (in rare exceptions, the mice were used in our colony). These mice include the following: IL-6−/− (24) on a B6 background; IL-1b−/− (25) on both B6 (5) and B6 129 F2 (6) backgrounds; TNF−/− (27) on a B6 129F2 background; Ltα−/− (31) on the B6 129F2 background (8); TNF-α−/− (22) on a B6 129F2 background; and TNFR1−/− (32) on a B6 129F2 background. The knockout mice used for serum transfer were obtained from the Jackson Laboratory, brought to our animal facility at the Harvard Medical School animal facility at 4–5 wk of age, and used 1–3 wk later (in rare exceptions, the mice were used in our colony). These mice include the following: IL-6−/− (24) on a B6 background; IL-1b−/− (25) on both B6 (5) and B6 129 F2 (6) backgrounds; TNF−/− (27) on a B6 129F2 background; Ltα−/− (31) on the B6 129F2 background (8); TNF-α−/− (22) on a B6 129F2 background; and TNFR1−/− (32) on a B6 129F2 background.
jected with a single dose of K/BxN serum, RNA was prepared at different times thereafter from ankle tissue (pooled from two individuals), and real-time PCR was performed to quantitate spliced TNF-, IL-1, and IL-6 mRNA transcripts. A representative experiment is shown in Fig. 1.

The first signs of induction were detectable a few hours after serum injection, with a modest but detectable rise from the baseline for all mRNA at 6 h. TNF mRNA increased more substantially from 24 h onwards. IL-1 transcripts followed roughly the same pattern, but with a sharper induction at 48 h and far more extensive induction, reaching 13,000-fold at maximum. IL-6 showed a delay, with a maximum by 72 h followed by a decline at 144 h that was reproducibly observed in several experiments. These results are consistent with an early appearance of inflammatory cytokines purely on the effector phase of the disease, unencumbered by their influences on the immunological induction phase. Such complications may have clouded results from collagen-induced arthritis (CIA) and antigen-induced arthritis models, where the known pleiotropic effects of such cytokines on the structure or responsiveness of the immune system complicate data interpretation. The K/BxN serum transfer system is applicable to a number of mouse strains (9), allowing one to investigate the effects of diverse natural and engineered mutations. This strategy was applied here, focusing on the contributions of IL-1, IL-6, and members of the TNF family, by transferring K/BxN serum into homogenous knockout 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 influences, with both local and systemic effects. For example, it promotes immune responses and plasma 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 transferred with serum from arthritic K/BxN mice, and arthritis development was compared as described previously (6). The representative experiment in Fig. 2A demonstrated a very similar arthritis course in IL-6-deficient and control mice. The initial onset of symptoms was the same, all distal joints were affected, and with a comparable degree of inflammation (as 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 image of synovitis and joint infiltration typical of K/BxN mice (synovial thickening and infiltration, presence of neutrophils in the articular cavity, pannus formation, cartilage destruction; Fig. 2C, unpublished data). Furthermore, cartilage damage and proteoglycan loss were evident on toluidine blue-stained ankle sections from serum-injected mice at comparable 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 induction phase of the CIA model: less intense immune responses were more likely to the collagen-II antigen in the absence of IL-6 function (29, 30). Together, then, the 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 targeting TNF, there is substantial body of evidence implicating this inflammatory cytokine in several
classic murine arthritis models, whether autoin mune in nature or induced by local microbial particles (32–36); simi-
larly, 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-1-α-elicited 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 lim in swelling of several digits and a slight fluctuation in ankle thickness (Fig. 3). To guard against possible influences of genetic background variability, we repeated the initial experiment performed in B6 (129)F2 mice in IL-1R–deficient and control mice. The results are summarized in Table I, allowing several conclusions.

These cut-out results indicate that, in this serum-transferred arthritis model mediated by arthritogenic Iggs, 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 importance 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 consis-
tent with the finding that intraarticular expression of IL-1, 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 inflam matory arthritis. This has ranged from the initial demonstration of TNF– expression in an animal model, to establishing the efficacy of TNF–/TNFR– blocking agents in animal models, to the successes of such reagents in therapeutic intervention in human RA (1, 4, 39–42). Absent expression of TNF– is also sufficient to induce arthritis in transgenic animals (43). These results evoked models of arthritogenesis in which TNF– 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– or its close homologue, lymphotoxin (LT–) (17–21). TNF– and LT– mediate their pleiotropic effects by binding to one of two known receptors, TNFR1 (p55) and TNFR2 (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 simply, LT– seemed not to be required for the development of K/BxN serum-transferred arthritis. LT––deficient mice responded normally on all counts, in the kinetics and intensity of inflammation and in the appearance of histological lesions (proliferative synovitis, infiltration of the joint cavity by neutrophils, and formative of a destructive pannus).

Second, the absence of TNF– had a marked effect in all of the models. Our standard fully susceptible background (B6 129)F2 mice in IL-1R–deficient and control mice (matched for gender/age and genetic background) were injected with 150 l serum from arthritic K/BxN mice on days 0 and 2. Arthritis was evaluated by measuring clinical index and ankle thickening as in Fig. 2. A) Data from a representative experiment, with each curve representing an individual mouse. B) Tabulation of the results for 10 knockout mice and 10 wild-type controls. (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.
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 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 129) F2 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−/− mice truly due to the absence of the cytokine, or instead to some independent factor (a linked gene effect, quite plausible given the genome 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 TNFR1 with an agonistic mAb. To test this prediction, we injected cohorts of TNF−/− 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 an arthritic effect, provoking arthritis in all the TNF−/− 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 mAbs with blocking or antagonist activity (55R-170, 55R-286). None of these reagents induced arthritis (Fig. 5B), at least not beyond the minority of TNF−/− 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−/− mice indeed have the element missing in TNF−/− mice that is required for robust development of arthritis.

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

<table>
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<th>Arthritis</th>
<th>Days of onset</th>
<th>Max CI</th>
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<tr>
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Figure 4. Variability of arthritis in TNF−/− mice. TNF−/− mice (left) and control mice (right; matched for gender/age and genetic background) were injected with 150 l serum from arthritic K/BxN animals (see Fig. 2). The data are pooled from six different experiments. All mice originated from the Jackson Laboratory.

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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 129) F2 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−/− mice truly due to the absence of the cytokine, or instead to some independent factor (a linked gene effect, quite plausible given the genome 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 TNFR1 with an agonistic mAb. To test this prediction, we injected cohorts of TNF−/− 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 an arthritic effect, provoking arthritis in all the TNF−/− 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 mAbs with blocking or antagonist activity (55R-170, 55R-286). None of these reagents induced arthritis (Fig. 5B), at least not beyond the minority of TNF−/− 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−/− mice indeed have the element missing in TNF−/− mice that is required for robust development of arthritis.
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 requiring a certain degree of local inflammatory insult, only seldom reached in the absence of TNF- . As the knockout mutation was carried on a mice background, we reasoned that mice bred at the Jackson Laboratory and shipped to Boston 7–15 d before challenge showed a mainly 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 one 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-mediated blockade. On the other hand, our results do concur with reports of induction of CIA in TNF- -deficient mice (46). Although it is conceivable that the cytokine network adapt-as described by TNF- in mice -- can occur in 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.

Environmental, not genetic, influences on TNF-independent arthritis. The significant mouse-to-mouse variability we have observed with TNF- -independent arthritis is, in a sense, reminiscent of the variability in the response of RA patients to TNF- /TNFR blockade (1). The results of Fig. 6 make it perhaps more plausible that environmental effects are at play, the degree of TNF- involvement being dependent on the general inflammatory state of the individual. It should be worth while trying to pinpoint what these influences might be, in both mice and human, and the present system provides a handle.

There are several potential interpretations for the strong arthritis that develops in TNF- /TNFR-deficient mice. The most straightforward idea is that other receptors can compensate and mediate TNF- signals. Although the existence of such a receptor has not been reported to date, the breadth of the TNF family makes it quite possible that other receptors will be found to bind TNF- . Whether these are indeed the primary receptors mediating arthritis, or whether they only come into play when the primary TNF FR1/2 receptors are absent, will need to be explored. Alternatively, one might propose that TNF- independent arthritis pathways are particularly active when TNF FR1/2 are missing, perhaps by contributing to downstream signal transduction adap-
tors. For example, the absence of TNF-1 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-kB 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-1 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-1, 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-1 was largely on par with the extent of inflammation.

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-1 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-1 in arthritogenesis. In animal models where the function of these cytokines has been tested, their importance varies somewhat with the disease-eliciting agent, although IL-1 may play a dominant 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 production of TNF-1 would be upstream of that of IL-1 (1). TNF-1 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-1 in promoting IL-1 production by synovocytes from RA patients (54), or with the fact that IL-1 blockade prevents the arthritis induced by transgene-encoded TNF-1 in mice (55). It should also be pointed out that the experiments shown in Fig. 1 only detect transcriptionally induced TNF-1 production. However, it is likely that even earlier release of TNF-1 occurs in the first minutes or hours of the disease, released from intracellular stores of synoviocytes or mast cells upon triggering by C5a or FcγR III. 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-1 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-1 release, a model bolstered by the
successes of anti-TNF- \( \theta \) therapy. The present results show that arthritis induced by Ab complexes in the joint also end up with the production of TNF- \( \theta \)- and IL-1, and is highly dependent on these cytokines.

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References


3. Firestein, G.S., and N.J. Zvaifler. 1990. How important are arthritis induced by Ab complexes in the joint also end up with the production of TNF- \( \theta \)- and IL-1, and is highly dependent on these cytokines.


27. Silacci, P., J.M. Dayer, A. D'Agostino, R. Peter, C. M. an-
ueddu, and P.A. Guerne. 1998. Interleukin (IL)-6 and its
soluble receptor induce TNF-P expression in synoviocytes and
chondrocytes, and block IL-1-induced collagenolytic activity.
28. Fattoni, E., M. Cappelletti, P. Costa, C. Sellitto, L. Cantoni,
M. Camilli, R. Foggiolini, G. Fantuzzi, P. Ghezzi, and V. Poli.
1994. Defective inflammatory response in interleukin 6-defi-
29. O'hishin, S., Y. Saike, T. Mima, M. Seaidi, K.N. Ishikoa,
N. Omura, M. Koki, Y. Koda, T. Tanaka, M. Suenaga, and
T.K. Ishino. 1998. Interleukin 6 plays a key role in the de-
USA. 95:8222–8226.
30. Takagi, N., M. Mihara, Y. Moriya, N. Nishimura, M. Ishi-
Blocking of interleukin-6 receptor ameliorates joint disease in
murine collagen-induced arthritis. Arthritis Rheum. 41:
2117–2121.
31. van de Loo, F., S. Kuiper, F.H. van Enckevort, O.J. Amtz,
and W.B. van den Berg. 1997. Interleukin-6 induces carti-
lage destruction during experimental arthritis. A study in in-
32. W. O'Conley, P.H., J.D. Haken, D.L. Cheon, A.E. Berger,
an interleukin-1 receptor antagonist protein on type II
collagen-induced arthritis and anti-tumor necrosis-induced ar-
33. van den Berg, W. B., L. Josten, M.M.A. Helser, and W.B. van
Den Berg. 1996. Anticytokine treat-
ment of established type II
collagen-induced arthritis in DBA/1 mice: a com-
1995. The type I interleukin-1 receptor acts as a nega-
tive or tumor necrosis factor (TNF) to induce arthritis in TNF-
35. J. O'Sullivan, M.M.A. Helser, F. van de Loo, and W.B. van
Den Berg. 1996. Antibody to type II collagen-induced ar-
inities in DBA/1 mice: a comparative study using anti-TNF-α,
anti-IL-1, and anti-IL-1. J. Immunol. 163:
297–309.
D. Heinegard, and W.B. van den Berg. 1999. IL-1 alpha beta
blockade prevents cartilage and bone destruction in murine
type II collagen-induced arthritis whereas TNF-α-alpha block-
ade only ameliorates joint inflammation. J. Immunol. 163:
5049–5055.
cytokines in rheumatoid arthritis. Annu. Rev. Immunol. 14:
397–440.
38. Ghirri, G., R. Kang, H.J. Goegegez, B.R. Leachan,
Suchaneck, L.R. M. C. Kenzle, et al. 1997. Constitutive intra-
cellular expression of human IL-1 beta following gene trans-
fer to rabbit synovium produces all major pathologies of hu-
Hardison, and M.A. Pallechino. 1992. Involved at 
endogenous type I tumor necrosis factor alpha and trans 
forming growth factor beta during induction of collagen type II arthritis in
40. Piguem, P.P., G.C.G. W, C. Vesin, H. Loetscher, R.G. entz,
mice accelerated by treatment with anti-tumor necrosis factor (TNF) or a recombinant soluble TNF receptor. Immunology. 77:510–514.
TNF F(ab')2 elicits joint disease in murine collagen-induced ar-
Influence of a recom binant human soluble TNF receptor Fc
fusion protein on type II collagen-induced arthritis in mice. J.
43. Keffer, J., L. Pinbert, H. Casaliers, S. Geogopoulou, E.
44. K. Keffer, J., L. Pinbert, H. Casaliers, S. Geogopoulou, E.
45. Ronan, T., J. Vilcek, and J.J. O'Connel. 1999. Program-
ming of cytokines in rheumatoid arthritis. Annu. Rev. Immunol. 17:
142–163.
47. K. Y. Y., J. Peace, J. Samol, B. Bobin, A. Tafel, M.O.
Activated T cells regulate bone loss and joint destruction in
Aron, 2001. The role of TNF-α receptor family members and
other TRAF-dependent receptors in bone resorption. Arth-
ritis Res. 3:6–12.
49. Kubotaka, K.N., T. Takahashi, E. Jimi, T. Udagawa, M. Takeni,
S. Katoke, N. Nagaoka, M. Kinosaki, K. Yamaguchi, N.
50. Jimi, E., K. Naka mu ra, L.T. Duong, T. Ikebe, N. Takahashi,
K. Ma no, and M. A. R. T. B. 1999. Involvement of osteoblasts/stromal cells in the
52. Brennan, F.M., D. Chantry, A. Jackson, R. Maini, and M.