11-2002

Critical roles for interleukin 1 and tumor necrosis factor alpha in antibody-induced arthritis

Hong Ji  
*Harvard Medical School*

Allison Pettit  
*Harvard Institutes of Medicine*

Koichiro Ohmura  
*Harvard Medical School*

*See next page for additional authors*

Follow this and additional works at: https://escholarship.umassmed.edu/rheumatology_pubs

Part of the [Immunopathology Commons](https://escholarship.umassmed.edu/immunopathology_commons), [Musculoskeletal Diseases Commons](https://escholarship.umassmed.edu/musculoskeletal_diseases_commons), [Rheumatology Commons](https://escholarship.umassmed.edu/rheumatology_commons), and the [Skin and Connective Tissue Diseases Commons](https://escholarship.umassmed.edu/skin_connective_tissue_diseases_commons)

Repository Citation

Ji, Hong; Pettit, Allison; Ohmura, Koichiro; Ortiz-Lopez, Adriana; Duchatelle, Veronique; Degott, Claude; Gravallese, Ellen M.; Mathis, Diane; and Benoist, Christophe, "Critical roles for interleukin 1 and tumor necrosis factor alpha in antibody-induced arthritis" (2002). *Rheumatology Publications and Presentations*. 35.  
https://escholarship.umassmed.edu/rheumatology_pubs/35

This material is brought to you by eScholarship@UMMS. It has been accepted for inclusion in Rheumatology Publications and Presentations by an authorized administrator of eScholarship@UMMS. For more information, please contact Lisa.Palmer@umassmed.edu.
Critical roles for interleukin 1 and tumor necrosis factor alpha in antibody-induced arthritis

Authors
Hong Ji, Allison Pettit, Koichiro Ohmura, Adriana Ortiz-Lopez, Veronique Duchatelle, Claude Degott, Ellen M. Gravallese, Diane Mathis, and Christophe Benoist

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

Publisher PDF posted as allowed by the publisher's author rights policy at http://www.rupress.org/site/subscriptions/terms.xhtml.

Creative Commons License
This work is licensed under a Creative Commons Attribution-Noncommercial-Share Alike 3.0 License.

Rights and Permissions
Citation: J Exp Med. 2002 Jul 1;196(1):77-85. doi: 10.1084/jem.20020439

This article is available at eScholarship@UMMS: https://escholarship.umassmed.edu/rheumatology_pubs/35
Critical Roles for Interleukin 1 and Tumor Necrosis Factor in Antibody-induced Arthritis

Hong Ji, Allison Pettit, Koichiro Ohmu ra, Adriana Ortiz-Lopez, Veronique Duchatelle, Claude Degott, Ellen Gravallese, Diane Mathis, and Christophe Benoist

Section on Immunology and Immunogenetics, Joslin Diabetes Center and Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA 02215
Institut de Genétique et de Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique/Institut National de la Santé et de la Recherche Médicale/Université Louis Pasteur, 67404 Strasbourg, France
Beth Israel Deaconess Medical Center and New England Baptist Bone and Joint Institute, Harvard Institutes of Medicine, Boston, MA 02215
Service d’Anatomie et de Cytologie Pathologiques, Hopital Beaujon, 92118 Clichy, France

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)-α was also required, although seemingly less critically than IL-1, because a proportion of TNF-α-deficient mice developed robust disease. There was no evidence for an important requirement for TNF-α for bone destruction. The variability in the requirement for TNF-α, 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 persisting 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 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-α and IL-1 is well established (4), out demographically by the immunosuppressive effects of therapeutic protocols that block TNF-TNF-R interactions (1). There has also been debate over the relative importance of the IL-1 and TNF-α 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-α blocking drugs.

*The present address of H. Ji is Serono Pharmaceutical Research Institute, 14 Chemin des Aulx, 1228, Plan-les-Ouates, Geneva, Switzerland.
Address correspondence to Diane Mathis and Christophe Benoist, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215. Phone: 617-264-2745; Fax: 617-264-2744; E-mail: cbdm@joslin.harvard.edu

Abbreviations used in this paper: CIA, collagen-induced arthritis; GPI, glucose-6-phosphate isomerase; LT, lymphotoxin; RA, rheumatoid arthritis.
The K/BxN TCR transgenic mouse is a recently developed model of inflammatory arthritis (5–9). All K/BxN animals spontaneously show an autoimmune disease with m orals (although not all of the clinical, histological, and immunological features of RA in hum ans. 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 autoimmune to a ubiquitously expressed antigen, glucose-6-phosphate isomerase (GPI). Transfer of anti-GPI IgGs from arthritic K/BxN mice into healthy mice also provokes arthritis 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 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 cell component and the primers of human joints is also of interest (10). Studies on K/BxN mice revealed augmented local synthesis of inflammatory cytokines, such as IL-6 and TNFα, 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 in arthritis (13).

Our early studies on K/BxN mice revealed augmented local synthesis of inflammatory cytokines, such as IL-6 and TNFα, 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 in inflammation appearing within 24 h in fully susceptible strains (9). To begin exploring the induction of various inflammatory cytokines, bone erosion, and cartilage destruction. An adjacent section was stained with toluidine blue (Sigma-Aldrich) for staining of cartilage surfaces of human joints is also of interest (10).

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 were involved in the following: IL-6 (14) on a B6 background; IL-1β (15) and B6 129/F2 (16) background; TNFα (17) on a B6 129/F2 background; Lta (18) on the B6 129/F2 background (19); TNFR1/2 (20) on a B6 background; TNFR1 (21) and TNFR2 (22) on a B6 background; Lta (23) on a (B6 × 129)F2 background. These mice include the following: IL-6 knockout mice, TNFR1/2 knockout mice, and TNFR1 knockout mice. These mice were used 1–3 wk later (in rare exceptions, the mice were bred in our colony). These mice also included the following: IL-6 knockout mice, TNFR1/2 knockout mice, and TNFR1 knockout mice. These mice were used 1–3 wk later (in rare exceptions, the mice were bred in our colony).

Inflammatory Cytokines in K/BxN Arthritis

RNA Analysis. RNA was prepared from ankle tissue by a modified LiCl/urea technique (22), designed to avoid contamination of the joint RNA with bone marrow-derived material by leaving the bone intact. A fixed section of ankles (six sections on the long bones of the lower leg and in the metatarsal area), the tissue was fixed in skin and superficial tendons. The joint was immersed in 1 ml RNA isolation solution (6 M urea, 2% SDS). Arthritic cavities were opened with a scalpel and were exposed to the medium to release the cellular contents. After 10 min incubation, the fragments were removed, and an equal volume of concentrated LiCl isolation (6 M LiCl, 6 M urea, and 10 mM sodium acetate, pH 5) was added to precipitate the RNA. RNA 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 were as follows: probe, 5’ CTTGGGCCGCTCCTTT TAMRA 3’; forward primer, 5’ CAGACGCACGTGCTGTTT 3’; and reverse primer, 5’ TGTCTTTGACCTTTTGACCATCCA 3’. For the quantification of TNFα and IL-6, the TaqMan predeveloped 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’ TCGACCTGGAGATGTG-GATTCCCTAMARA 3’; forward primer, 5’ TGAAGACGGCACCACCA 3’; and reverse primer, 5’ AAGCCGTCTTTC-CATCTCTCTCT 3’. To determine the relative expression values, Cβ (cyclophilin), Cα (cytokine) Cβ (cytochrome c) was used to derive an expression index (24), which was then divided by the same index obtained with a reference sample of total somatic RNA.

Serum Transfer Protocol and Arthritis Scoring. K/BxN serum pools were prepared from arthritic mice 60 d old. A arthritis was induced by intraperitoneal injection of 150–200 l serum at days 0 and 2. A clinical index was established (1) for each affected paw: 0.5 points for a paw with only mild swelling/redness or only a few digits affected. Arthritis was assessed by a caliper (6), with ankle thickening being defined as the difference in ankle thickness from the day 0 measure.

Histology. Hind limb bones were collected and the knee and ankle joints were separated in bid-chia. Specimen was dissected to reveal skin and muscle, and subsequently fixed in 4% paraformaldehyde for a minimum of 12 h and demineralized for 2 wk in 14% EDTA, followed by paraffin-embedded sections (100; Shandon). For each specimen, twenty 4-μm sagittal serial sections were cut, and every fifth section was stained with hematoxylin and eosin. Six signs were described previously (6, 23).
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-α, 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 an odour that was detectable in the first RNA sample at 6 h. TNF mRNA increased m ore substantially from 24 h onwards. IL-1 transcripts followed roughly the same pattern, but with a sharper induction. The induction of IL-1 appears somewhat more extensive from 24 h onwards. IL-6 showed a delay, with a more substantial induction. The induction of IL-1 appears somewhat more extensive from 24 h onwards. IL-6 showed a delay, with a more substantial induction. The induction of IL-1 appears somewhat more extensive from 24 h onwards. IL-6 showed a delay, with a more substantial induction. The induction of IL-1 appears somewhat more extensive from 24 h onwards.
Published July 1, 2002

Figure 2. No requirement for IL-6 in arthritis induced by K/BxN serum transfer. IL-6-deficient and control mice were injected with 150 μl serum from arthritic K/BxN mice on days 0 and 2. A) The results were evaluated by measuring clinical index and ankle thickening. M and M(t) represent the 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 for Fig. 2; the star denotes a transient inflammatory reaction in the digits of one mouse.

Figure 3. Essential role of IL-1. IL-1R–deficient and control mice were injected with 150 μl 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 in B6 recipients, 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 129F2 background or on an inbred B6 background. Scoring was described for Fig. 2; the star denotes a transient inflammatory reaction in the digits of one mouse.

Inflammatory Cytokines in K/BxN Arthritis

The development of K/BxN serum-transferred arthritis is reminiscent of its requirement in CIA and other murine arthritis models (32, 33, 35). It is also consistent with the finding that interleukin-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 inflammatory arthritis. This has ranged from the initial demonstration of TNF– expression in arthritic synovium, to establishing the efficacy of TNF–/– or TNFR– mice in animal models, to the successes of such reagents in therapeutic intervention in human RA (1, 4, 39–42). Aberrant expression of TNF– is also sufficient to induce arthritis in transgenic animals (43). These results evolved 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– mice exhibit pleiotropic effects by blocking the 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 respond 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 formation of a destructive pannus).

Second, the absence of TNF– had a more profound effect on the arthritogenicity of K/BxN serum. Any TNF– deficient mice developed no disease whatsoever upon transfer of K/BxN serum, either clinically or histologically (Table I). However, a number of such animals did develop joint inflammation, overall in 9/...
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 confirmed 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-α, with no reported indication of another possible receptor in spite of the broad attention that TNF-α 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-α mutation: was the poor responsiveness in TNF-α-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-α replacement, e.g., by triggering TNFR1 with an agonistic mAb. To test this prediction, we injected cohorts of TNF-α-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-α-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 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-α-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-α is indeed the element missing in TNF-α-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>
<td>4, 2, 2, 1, 1, 2, 2</td>
<td>8/8</td>
<td>3, 4, 4, 4, 4, 4, 4, 3</td>
</tr>
<tr>
<td>TNFR2/8/8 (B6)</td>
<td>4, 2, 2, 1, 1, 1, 2, 2</td>
<td>8/8</td>
<td>3, 4, 4, 4, 4, 4, 3</td>
</tr>
<tr>
<td>TNFR1/2 (B6x129F2)</td>
<td>2, 1, 4, 2, 2</td>
<td>6/6</td>
<td>4, 4, 4, 15, 35, 3</td>
</tr>
<tr>
<td>Lt (B6x129F2)</td>
<td>2, 2, 2, 2, 2, 1, 4, 2</td>
<td>8/8</td>
<td>4, 4, 4, 4, 2, 4, 3</td>
</tr>
<tr>
<td>TNF/9/9 (B6x129F2)</td>
<td>2, 2, 2, 3, 5, 4, 2, 3</td>
<td>9/9</td>
<td>2, 5, 3, 4, 2, 3, 5</td>
</tr>
</tbody>
</table>

Figure 4. Variability of arthritis in TNF-α-deficient mice. TNF-α-deficient (left) and control mice (right; matched for gender/age and genetic background) were injected with 150 l serum from arthritic K/BxN animals on days 0 and 2. Arthritis was assessed by measuring ankle thickening as in Fig. 2. The data are pooled from six different experiments. All mice originated from the Jackson Laboratory.

Published July 1, 2002
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 or stochastic threshold effects could also be involved. Arthritisogenesis involving a certain degree of local inflam matory insult, only seldom reached in the absence of TNF- . As the knockout mutation was carried on a m i xed (129x8) F2 background, we reasoned that m odifier 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 m ice. Should alleles at independent loci be segregating, there should be heritable transmission of these traits to the progeny. A s shown in Fig. 6 A, this was not the case. A cross between two resistant m ice yielded a dominant proportion of responder m ice; the transmission of a recessive susceptibility allele in this family would be very unlikely to yield such a pattern (P  0.003). Thus, the variability does not stem from M endelian genetic elements. Epigenetic variation could perhaps account for these results. How ever, we observed a clear correlation between the origin and life history of the m ice and their responses to K/BxN serum (Fig. 6 B). Those m ice 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 m ouse pathogens, but minor bacterial flora varies. Thus, the segregation of responses is one consistent with an environment explanation 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 m ice. We have also made similar observations, injecting several different anti-TNF-- reagents into young K/BxN m ice (unpublished data). However, we interpret these negative results with caution because of the very aggressive nature of the disease that develops in the transgenic m ice and uncertainties concerning the efficiency of Ab-mediated blockade. On the other hand, the present results do concord with reports of robust development of CIA in TNF----deficient m ice (46). Although it is conceivable that the cytokine network adapts somewhat in TNF----deficient m ouse, the variability in the response of RA patients to TNF--/ TNFR blockade (1). The results of Fig. 6 make it perhaps more plausible that different factors play, the degree of TNF-- involvement being dependent on the general inflammatory state of the individual. It should be worthwhile trying to pinpoint whether these influences might be, in both m ice and human, and the present system does provide a handle.

There are several potential interpretations for the strong arthritis that develops in TNF----deficient m ice. The most straightforward 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 TNFR 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 TNFR1/2 receptors are absent, will need to be explored. Alternatively, one might propose that TNF----independent arthritis pathways are particularly active when TNF FR 1/2 are missing, perhaps by complementing downstream signal transduction adap-

Figure 5. Triggering the TNF receptor complements TNF-- deficiency. TNF----deficient m ice were injected with 150 lassa from arthritis K/BxN mice on days 0 and 2. A. Anti-herpes virus antibodies, few signs of disease by day 7 were injected at days 7, 11, and 15 with anti-TNF FR 1 m Abs 55R--293, which has significant agonist activity (A) or with control m Abs (B). These controls included anti-TNF FR 1 m Abs devoid of agonist activity or an irrelevant m Ab. C) Anti-TNF FR m Abs were injected without K/BxN serum. A threshold is assessed by measuring ankle thickening above. The data are pooled from four different experiments and m ices originated from the Jackson Laboratory.

Figure 6. Environmental, not genetic, influences on TNF----independent arthritis. A) TNF----deficient m ice from the Jackson Laboratory were tested by transfer of K/BxN serum, and m ices of different phenotypes were crossed. White spots indicate resistant m ices; black spots indicate susceptible m ices, where resistance and susceptibility are defined as the presence of clear arthritis (grade 1) in the first 10 d after serum transfer. Their progeny was similarly tested when 4–5 wk old. B) A compilation of results of challenge of TNF----deficient m ices with K/BxN serum, either from m ices purchased from the Jackson Laboratory or bred in our Boston colony, *P  0.003.
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 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 possible 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 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− 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 joint presence varies as to whether they are disease-inducing agents, 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 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 from RA patients (54), or with the fact that IL-1 blockade prevents the arthritis induced by transgene-encoded TNF− misexpression (34). 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 synoviocytes or mast cells upon triggering by C5a or FcR 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− 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

**Figure 7.** Bone destruction in control and KO mice. Bone erosion was assessed in hematoxylin and eosin-stained ankle sections from TNF− and LT−deficient mice and control mice after transfer of K/BxN serum. (A) Control mouse, full inflammation and areas of focal bone destruction (arrows). (B) Arthritic LT−deficient mouse with inflammation and focal bone erosion. (C) TNF−deficient mouse with clinical manifestations, showing inflammation and focal bone erosion. (D) TNF−deficient mouse with no clinically detectable symptoms, showing minimal inflammation (matched with A).
sucsesess of anti-TNF - therapy. The present results show that arthritis induced by Ab complexes in the joint also end up w th the production of TNF and IL-1, and is highly dependent on these cytokines.

We would like to thank D. M. R. Schreiber for the generous gift of mAbs, and J. H. esqueux, S. Johnson, and Q. M. Pham for excellently managing the KRN colony.

This work was supported by grants from the Association pour la Recherche contre la Polyarthrite and the National Institutes of Health (IR 01 AR/A16680-01) to D. M. asian and C. Benoist, and an Arthritis Foundation Biomedical Science grant to E. G. Neville.

K. O. h m wa received a fellowship from the Uehara Foundation, and A. Pettit is supported by the National Health and Medical Research Council of Australia, and by the Arthritis Foundation.

Submitted: 19 March 2002
Accepted: 14 May 2002

References


Downloaded from jemp.rupress.org on March 15, 2015

159:3364–3371.
27. Silacci, P., JM. Dayer, A. Degeorges, R. Peter, C. M. an-
ueddu, and P.A. Gue neur. 1998. Interleukin (IL)-6 and disso-
vable receptor induce TMP-1 expression in synovocytes and
chondrocytes, and block IL-1-induced collagenolytic activity.
28. Fantoni, B., M. Cappelletti, P. Costa, C. Selitto, L. Cantoni,
M. Caelli, R. Faggioni, G. Fantuzzi, P. Ghedizzi, and V. Poli.
1994. Defective inflammatory response in interleukin 6-defici-
N. omaza, M. Kofu, Y. Kato, T. Tanka, M. Suen uza, and
T. Kishimoto. 1998. Interleukin 6 plays a key role in the de-
USA. 95:8222–8226.
30. Takagi, N., M. Mihara, Y. Moriya, N. Nishimoto, K.
Takagi, N., M. Mihara, Y. Moriya, N. Nishimoto, K.
31. van de Loo, F.A., S. Kuiper, F.H. van Enckevort, O.J. Arntz,
t. Saxne, F.A. van de Loo, D.
32. W ooley, P.H., J.D. Wh a len, D.L. Cha pman, A.E. Berger,
33. Joosten, L., M.M.A. He lsen, F. van de Loo, and W .B. van
34. Johansen, S., C. Vestin, H. Loetscher, R. G. entz,
38. Hardyson, and M.A. Palladino. 1992. Involvem ent of endog-
40. Pikuet, P.P., G.E. G iau, C. Vestin, H. Loetscher, R. G. entz,
43. Keffer, J., L. Pinbert, H. Casaliers, S. Geogopoulos, E.
44. Krakauer, T., J. Vilcek, and J.J. O ppenheim. 1999. Proin-
45. L. Mc Ke nzie. 1994. Defective inflammatory response in interleukin 6-defi-
48. Gravallese, E.M., D.L. Golberg, and P.E. Au-