Mast cells contribute to initiation of autoantibody-mediated arthritis via IL-1

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Mast cells are immune sentinels that participate in the defense against bacteria and parasites. Resident within the joint, mast cells become activated in human rheumatoid arthritis and are implicated in the pathogenesis of experimental murine synovitis. However, their arthritogenic role remains undefined. Using a model of autoantibody-induced arthritis, we show that mast cells contribute to the initiation of inflammation within the joint by elaboration of IL-1. Mast cells become activated to produce this cytokine via the IgG immune complex receptor FcγRIII. Interestingly, mast cells become dispensable for the perpetuation of arthritis after delivery of IL-1, highlighting the contribution of this lineage to arthritis induction. These findings illuminate a mechanism by which mast cells can participate in the pathogenesis of autoimmune inflammatory arthritis and provide insights of potential relevance to human rheumatoid arthritis.

cytokine | mouse model | synovitis

Rheumatoid arthritis (RA) is a destructive inflammatory disease of the joints affecting up to 1% of the population (1). Although disease pathogenesis remains obscure, evidence implicates T and B cells as well as innate immune elements (2). Recently, therapeutic success with B cell depletion and appreciation of the diagnostic utility of antibodies against citrullinated peptides has renewed interest in the role of antibodies in this disease, highlighting previous research on the prevalence of circulating and synovial immune complexes as well as abnormal IgG glycosylation in patients with RA (3–6).

To understand the mechanisms whereby antibodies may cause arthritis, attention has turned to animal models of autoantibody-mediated joint disease. One of these is the K/BxN model, which shares with RA a dependence on T cells, B cells, and certain cytokines as well as clinical features, including symmetry, a distal-to-proximal gradient of severity, and the formation of erosive pannus within the joint (7). K/BxN arthritis results from polyclonal IgG antibodies against the autoantigen glucose-6-phosphate isomerase, whose pathogenic effector pathways include the C5a receptor (CD88), the IgG receptor FcγRIII, and the IL-1 receptor IL-1R1 (8–12). TNF can contribute but is not absolutely required. At the cellular level, depletion of neutrophils abrogates disease. Strikingly similar pathogenic pathways characterize other murine models of arthritis, including collagen-induced arthritis, suggesting that these mechanisms reflect a general pathway to arthritis (13).

Recently, studies in the K/BxN model have demonstrated a pathogenic contribution by mast cells (14–16). Mast-cell-deficient mice (W/W^v and Sl/Sl^d) are resistant to arthritis, whereas engraftment of W/W^v animals with wild-type mast cells restores susceptibility. However, the pathways by which mast cells participate in antibody-mediated arthritis remain incompletely defined.

Investigating the role of the mast cell in K/BxN serum-transfer arthritis, we found that a brief course of exogenous IL-1 could bypass the requirement for mast cells in W/W^v mice. Engraftment of cultured IL-$1^{-/-}$ mast cells into these mast-cell-deficient animals confirmed that mast cells are an obligate source of this cytokine in our experimental system. Interestingly, although arthritis in IL-$1^{-/-}$ mice provided with a pulse of exogenous IL-1 was transient, arthritis in mast-cell-deficient W/W^v mice treated similarly exhibited a normal course, highlighting the contribution of mast cells in disease initiation. Using *in vitro* studies and *in vivo* reconstitution experiments, we show that production of IL-1 by mast cells results from ligation of FcγRIII. Together, these findings demonstrate that mast cells can provide a "jump start" for IgG-mediated inflammatory joint disease via IL-1.

Results

Essential Role of IL-1 in K/BxN Arthritis. In other models of inflammation initiated via mast cells, TNF has emerged as the cytokine of critical importance (17–19). However, a substantial fraction of TNF-null animals administered K/BxN serum develop arthritis (12), implying that other mast cell mediators are operative in this context. Because previous work has shown that IL-1R1-null animals are resistant to K/BxN arthritis (12), we turned our attention to IL-1 as a proinflammatory cytokine of potential mast cell origin (20). Using animals genetically deficient in IL-1 (21), we confirmed that serum-transferred arthritis exhibits a striking dependence on IL-1 (Fig. 1 *A–D*). Furthermore, a brief course of IL-1β together with arthritogenic K/BxN serum restored robust, albeit transient, arthritis in the mutant animals (Fig. 1*E*). This finding defines an ongoing role for IL-1 in synovitis and demonstrates the ability of recombinant IL-1β to substitute functionally for endogenous IL-1 in supporting joint inflammation.

Mast Cells Are an Obligate Source of IL-1 in K/BxN Arthritis in W/W^v Mice. Hypothesizing that mast cell elaboration of IL-1 was an important event, we reasoned that administration of IL-1 might restore arthritis sensitivity to mast-cell-deficient W/W^v mice (14). As shown in Fig. 2*A*, a short course of IL-1 could fully complement mast cell deficiency in W/W^v animals and restore a normal disease course; IL-1 administered in the absence of serum resulted in no arthritis ($n = 8$ in two experiments; data not shown). Histologic evaluation for the presence of mast cells

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Abbreviation: RA, rheumatoid arthritis.

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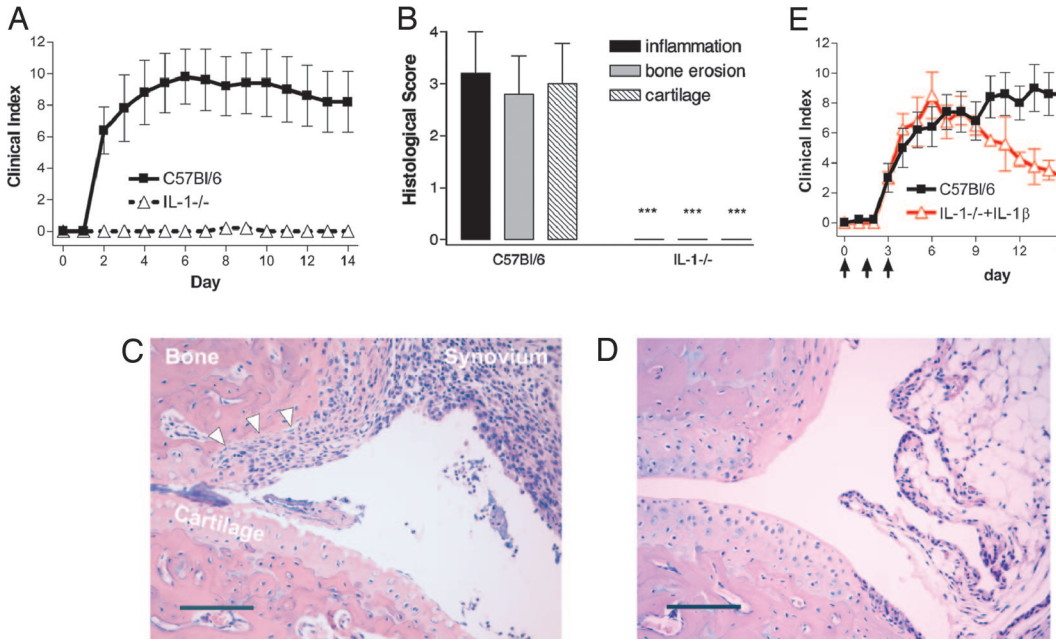

Fig. 1. IL-1 restores K/BxN arthritis susceptibility to IL-1-deficient mice. (*A*) Wild-type vs. IL-1$^{-/-}$ (n = 5 mice per group; $P < 0.001$). The results shown are representative of three experiments. (*B*) Histomorphometric quantitation of ankle tissues (n = 5 mice per group, ***, $P < 0.001$). (*C* and *D*) Representative ankle sections from wild-type (*C*) and IL-1$^{-/-}$ (*D*) mice. Arrowheads indicate erosions. (Scale bars, 100 μm.) (*E*) K/BxN serum administered to wild-type or IL-1$^{-/-}$ animals also given IL-1β (arrows) (n = 4 or 5 mice per group; $P < 0.001$). The results shown are representative of three experiments. All error bars indicate SEM.

remained negative in all W/W^v animals throughout the experiment. Because mast cells may contain preformed TNF (22) and because they participate in initiation of inflammation in other models via elaboration of this cytokine, we tested the ability of recombinant TNF to bypass the requirement for mast cells in arthritis-resistant W/W^v mice. Unlike IL-1, TNF administered at or significantly above doses that effectively complement mast cell deficiency, modulate systemic lymphocyte activation, and induce adhesion molecule expression on distant endothelium (18, 23, 24) could not complement mast cell deficiency in W/W^v mice (Fig. 2*B*). To further demonstrate the specificity of this IL-1 reconstitution effect, we assessed the ability of IL-1 to induce arthritis in mast-cell-sufficient CD88 (C5aR)-null mice (11). Administration of IL-1 failed to restore arthritis susceptibility in CD88$^{-/-}$ mice, illustrating that IL-1 cannot reverse all causes of resistance to K/BxN serum transfer arthritis (Fig. 2*C*).

To directly establish that mast cells are indeed an obligate source of IL-1 in K/BxN serum-transfer arthritis induction, we used a genetic approach. Wild-type and IL-1$^{-/-}$ bone-marrow-derived mast cells (BMMC) were engrafted into W/W^v mice in which K/BxN serum was administered to induce arthritis (14). As shown in Fig. 3 *A* and *B*, mock-engrafted W/W^v mice were resistant to arthritis, whereas mice engrafted with wild-type mast cells developed arthritis similarly to WBB6 littermate controls. In contrast with wild-type mast cells, IL-1$^{-/-}$ mast cells could not promote arthritis, despite synovial mast cell engraftment equivalent to wild-type BMMC (Fig. 3 *C* and *D*). These results confirm that mast cells can contribute to disease induction via elaboration of IL-1.

Evidence That IL-1 Is Produced by Mast Cells at the Site of Inflammation. Where do the mast cells responsible for IL-1 elaboration reside? Our group has previously demonstrated that K/BxN serum-induced degranulation of mast cells is observed in the joints and not at other anatomic sites (14). This result is consistent with the absence of pathology outside the joints in K/BxN serum-transferred arthritis (8) and may be related to the joint specificity of serum-induced vascular leak (25). However, as previously reported (26),

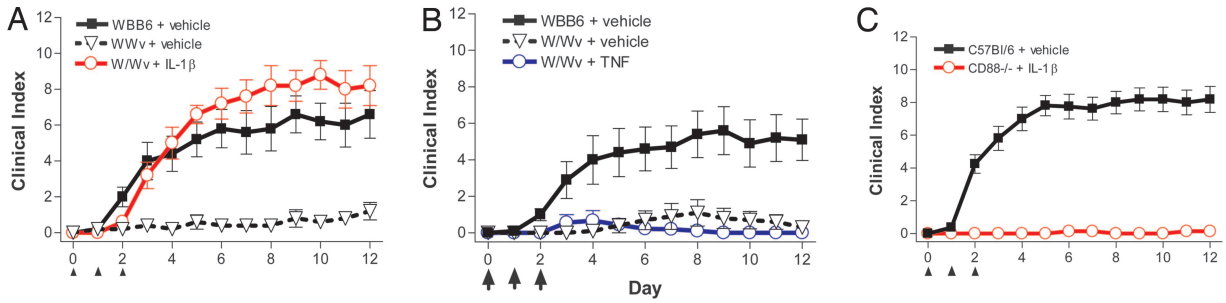

Fig. 2. Exogenous IL-1 restores susceptibility to arthritis in mast-cell-deficient mice. (*A*) W/W^v and control mice administered K/BxN serum and either IL-1β or vehicle (black arrows) (n = 5 mice per group). The data shown are representative of two experiments ($P < 0.001$ for W/Wv and IL-1β vs. vehicle; P was not significant vs. WBB6). (*B*) W/W^v and control mice administered K/BxN serum and 2.5 μg of TNF (n = 5), 7.5 μg of TNF (n = 4), or vehicle (black arrows). The data shown were pooled from two experiments ($P < 0.001$). (*C*) Administration of K/BxN plus IL-1β (arrows) to C5aR$^{-/-}$ mice. The results shown were pooled from two experiments (n = 16 B6 and 7 C5aR$^{-/-}$ animals; $P < 0.001$). All error bars indicate SEM.

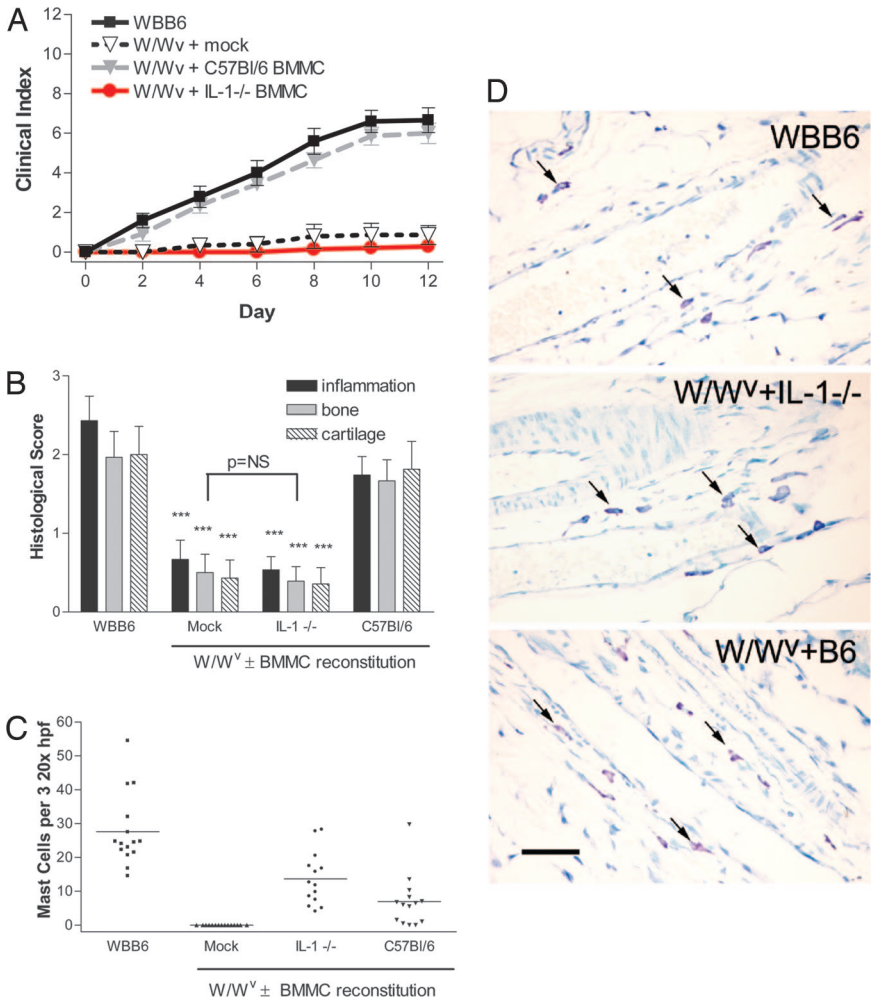

Fig. 3. Mast cells participate in arthritis via elaboration of IL-1. (*A*) Four-week-old W/W^v mice were engrafted with B6- or IL-1-deficient BMMC and administered K/BxN serum. Error bars indicate SEM. The results shown were pooled from three experiments (n = 14–15 per group; $P < 0.001$, IL-1$^{-/-}$ vs. B6 engraftment). (*B*) Histologic scoring of arthritis. ***, $P < 0.001$. (*C*) Efficacy of synovial mast cell engraftment (*P* not significant, IL-1$^{-/-}$ vs. B6). (*D*) Engrafted B6 and IL-1$^{-/-}$ synovial mast cells exhibit normal morphology and distribution. Arrows indicate mast cells; V, vessel. (Scale bar, 50 μm.)

mast cells abnormally populate the spleens of W/W^v mice reconstituted with BMMC, providing a potential source of systemic IL-1 that could confound the interpretation of our engraftment experiments. To address this possibility, we injected engrafted and unengrafted W/W^v mice with K/BxN serum and harvested serum and splenic tissue 4.5 h later, a time at which exogenous IL-1 (where administered) was readily detectable (Fig. 4*A*). Irrespective of engraftment status, administration of K/BxN serum did not result in significant systemic levels of IL-1 in any mouse at this time point (Fig. 4*A*). Furthermore, splenic IL-1 protein and mRNA levels provided no evidence that mast cells in the spleen were being activated to generate IL-1, despite a >100-fold excess of splenic mast cells in reconstituted W/W^v animals compared with controls (Fig. 4 *B–D*). By contrast, mice treated with i.p. LPS or with K/BxN serum followed 4 h later by IL-1, as per our IL-1 repletion protocol, demonstrated prominent levels of systemic and splenic IL-1.

To further confirm the irrelevance of splenic mast cells, we performed splenectomies on BMMC-engrafted W/W^v mice and found that splenectomized and sham-splenectomized animals demonstrated similar susceptibility to arthritis (Fig. 4*E*). We conclude that splenic mastocytosis does not confound the interpretation of the W/W^v engraftment experiments. Rather, absence of measurable circulating IL-1 supports the hypothesis that mast cells local to the joint are the relevant source of this cytokine in synovial inflammation.

Mast Cells Elaborate IL-1 upon Stimulation via Fcγ Receptors. If IL-1 from synovial mast cells is a key mediator in arthritis, how do anti-glucose-6-phosphate isomerase antibodies induce production of this cytokine? The demonstration of circulating and joint-resident immune complexes as well as the genetic requirement for C5aR and FcγRIII all point to the role of immune complexes in disease initiation (15, 27–29). Murine mast cells express FcγRIII and may be stimulated to degranulate and elaborate eicosanoids via this receptor in culture, although activation is constrained by the inhibitory receptor FcγRII (30, 31). To examine the capacity of mast cells to produce IL-1 upon specific ligation of FcγRIII, we generated BMMC from FcγRII$^{-/-}$ mice (32). Immune complex stimulation was mimicked by mAb cross-linking of FcγRIII. As shown in Fig. 5*A*, FcγRII$^{-/-}$ BMMC responded to FcγRIII ligation with brisk degranulation and production of IL-1.

To confirm an *in vivo* role for IgG Fc receptor-mediated mast cell activation in this model, we engrafted mast cells deficient in Fc receptors (FcR$\gamma^{-/-}$) into W/W^v mice (33). Despite synovial engraftment to equivalence with wild-type BMMC, these mast cells proved unable to confer arthritis [Fig. 5*B* and

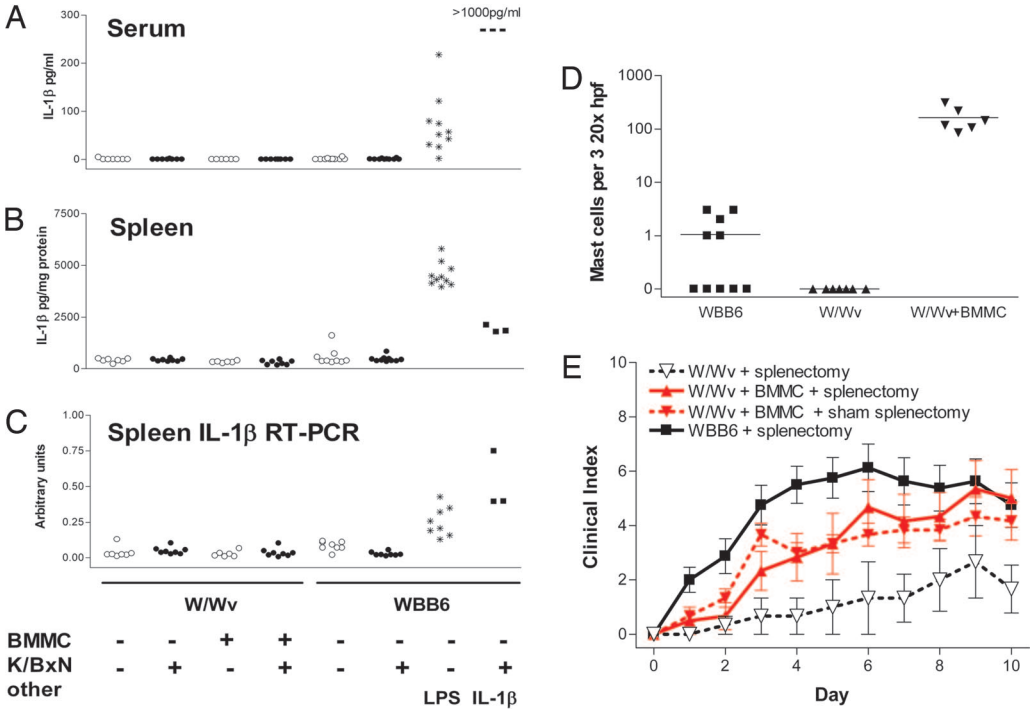

Fig. 4. Splenic mast cells do not participate in the initiation of arthritis. (*A–C*) Serum (*A*) and splenic (*B* and *C*) IL-1β production 4.5 h after administration of 150 μl of $\times$1 K/BxN serum or 50 μg of LPS. Where administered, IL-1β (2.5 μg) was given 4 h after serum injection ($n = 3$; otherwise, $n = 6$–10 mice per group pooled from two experiments). (*D*) Quantitation of splenic mast cells. (*E*) BMMC engrafted (15–16 weeks) W/W^v and control mice were treated with splenectomy or sham splenectomy and allowed 2–3 weeks of recovery time before arthritis induction (*P* not significant, W/W^v plus BMMC plus sham vs. splenectomy). Error bars indicate SEM ($n = 5$–8 per group and $n = 3$ W/W^v unengrafted animals).

supporting information (SI) Fig. 6]. The FcRγ chain is shared by FcεRI, FcγRI, FcγRIII, and the recently described FcγRIV (34). Previous genetic studies have found that FcγRI is dispensable for arthritis induction, whereas *in vitro* assays demonstrate that FcγRIV does not bind the IgG1 subclass responsible for disease in the K/BxN model (7, 11, 34). To exclude participation

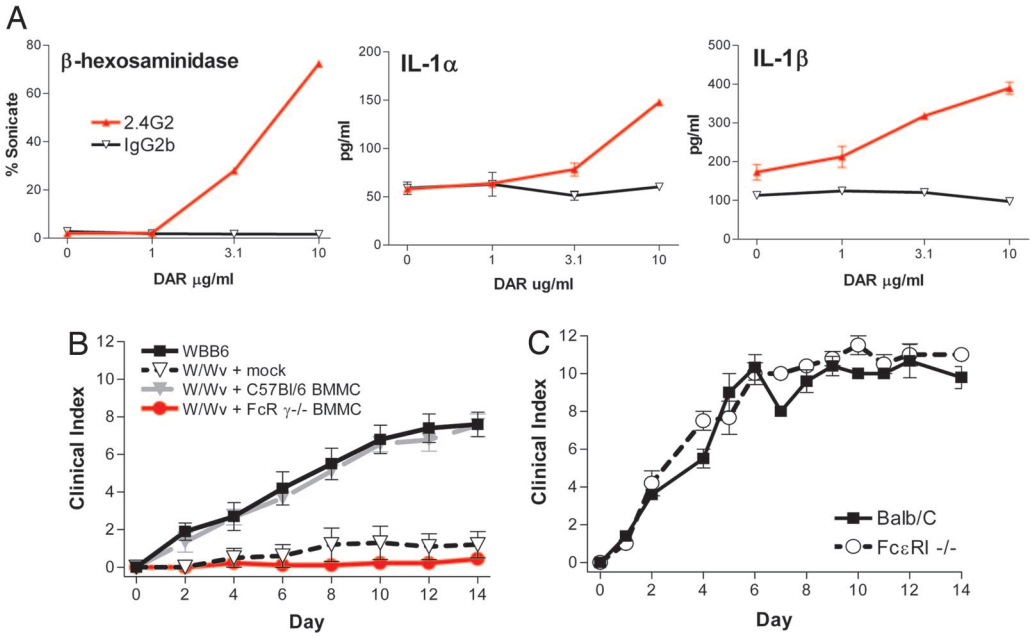

Fig. 5. Mast-cell production of IL-1 is mediated by FcγRIII. (*A*) FcγRII-null BMMC incubated with anti-FcγRII/III antibody 2.4G2 or isotype control followed by cross-linking donkey anti-rat (DAR) F(ab′)2. Results are the mean from duplicate wells and are representative of three independent experiments. (*B*) W/W^v mice engrafted with BMMC from B6- and FcγR-null mice and administered arthritogenic K/BxN serum. The results shown were pooled from two experiments ($n = 10$ mice per group; $P < 0.001$, B6 vs. FcγR$^{-/-}$ BMMC engrafted). (*C*) FcεRI-deficient and control BALB/c mice administered K/BxN serum (100 μl per injection). The results shown were pooled from two experiments ($n = 5$ mice per group; *P* not significant). All error bars indicate SEM.

of FcεRI in arthritis pathogenesis, we administered K/BxN serum to mice deficient in the FcεRI α chain (35). These animals exhibited wild-type susceptibility to disease (Fig. 5*C*). Thus, consistent with our *in vitro* findings, these data confirm that mast cell dysfunction derives from lack of expression of FcγRIII, the IgG immune complex receptor shown previously to be critical for disease pathogenesis (11, 15, 29).

Discussion

Although many aspects of the etiology of RA remain obscure, substantial evidence has accumulated that antibodies contribute to disease pathogenesis. In seropositive RA, unlike in other inflammatory arthritides, such as gout or psoriatic arthritis, deposition of immune complexes is typically observed in synovium and cartilage, whereas joint effusions exhibit prominent consumption of complement (5, 36, 37). Similarly, circulating and synovial fluid immune complexes may be demonstrated in the majority of patients (5, 38). Intriguingly, autoantibodies are often present years before the onset of arthritis (39). What events convert a patient with serologic abnormalities into one with clinical disease? Using a mouse model of autoantibody-induced arthritis, we here demonstrate one potential pathway: immune complex stimulation of mast cells via a low-affinity IgG Fc receptor results in the elaboration of IL-1, potentiating the initiation of inflammation within the joint by pathogenic autoantibodies. If this role is bypassed, such as by administration of exogenous IL-1, mast cell deficiency no longer impedes the development of robust synovitis. Together, these results define a functional mast cell contribution to the initiation of autoimmune inflammation within the joint and identify IL-1 as a mast cell effector function in this activity.

This work further develops the understanding of the mast cell as immune sentinel (40). Mast cells are found constitutively in most tissues, clustered at sites where foreign invaders are first encountered, such as the skin, mucosal surfaces, and around blood vessels. They also reside proximate to vulnerable body cavities, such as the peritoneum and joint (41). Although equipped with a broad range of antimicrobial effector mechanisms, a major role of mast cells appears to be the mobilization of leukocytes and other immune elements upon the first signs of infection, a purpose for which they are uniquely suited given their capacity for rapid release of preformed and newly synthesized mediators (42). We postulate that the involvement of mast cells in arthritis represents a "down side" of this physiologic specialization: Monitoring the joint for bacterial invasion of the protein-rich but acellular and hypocomplementemic synovial fluid (36), mast cells may instead become activated by autoantibody-containing immune complexes, thereby participating in the pathogenesis of destructive synovitis.

These findings define a clear example of IgG-driven mast cell participation in organ-specific autoimmunity, extending earlier work on the activation of mast cells via Fcγ receptors in IgG-mediated anaphylaxis and cutaneous immune complex disease (31, 32, 43, 44). Although we find that similar mechanisms for mast cell activation are operative in the joint, we herein offer *in vivo* demonstration of a disease-provoking effector mechanism, IL-1 elaboration, downstream of FcγRIII-mediated mast cell activation. Although IL-1 is well appreciated as an important mediator in the pathogenesis of arthritis, the key source for this cytokine within the joint has generally been thought to be the synovial macrophage (45, 46). Although mast cells belong to the myeloid lineage, it was unexpected to find that they should represent an important source of IL-1 in arthritis. Taken together, these results demonstrate a role for mast cells in the chain of events linking adaptive immunity and the production of this potent innate proinflammatory mediator.

Our conclusions, in common with much work defining *in vivo* mast cell function, is derived from observations in BMMC-reconstituted W/W^v animals (47). Impaired c-*kit* signaling in this strain has effects beyond the mast cell lineage, and it may be that the activity of the mast cell emerges with particular clarity in this genetic context. Recently, we have found that another mouse strain lacking mature tissue mast cells, C57BL/6-*Kit*$^{W\text{-}sh}$/ *Kit*$^{W\text{-}sh}$ (W^{sh}), that bears an inversion encompassing regulatory elements that modulate the tissue-specific expression of *c-kit* (48, 49) is susceptible to K/BxN serum-transfer arthritis, unlike the mast-cell-deficient W/W^v and Sl/Sl^d strains (P.A.N. and D.M.L., unpublished data). In preliminary experiments, we find that there exist cells in W^{sh} animals that are functionally able to provide the arthritogenic activity supplied by mast cells in engrafted W/W^v mice. Experiments designed to uncover the differences between these strains may yield important insights into mast cell physiology.

In light of discordance among mast-cell-deficient strains, it was especially important to consider the possibility that the apparent mast-cell-dependence of arthritis in reconstituted W/W^v mice might be an artifact of IL-1-producing splenic mast cells that have no counterpart in normal physiology. However, an intact arthritic response in engrafted and splenectomized W/W^v mice demonstrates that accumulated splenic mast cells play no discernable role in arthritis susceptibility (Fig. 4). Moreover, because administration of K/BxN serum does not result in measurable quantities of circulating IL-1, our data are consistent with a primary contribution by mast cells resident locally to the joint.

Our data highlight the importance of mast cells in the early phase of disease, yet this is unlikely to represent the whole story of mast cells in arthritis. In the model used in these studies, an initial pulse of arthritogenic autoantibodies results in a monophasic illness (8). By contrast, the generation of autoantibodies in RA is ongoing, and the disease runs a chronic course punctuated by recurrent flares. Whereas fluid from active joints commonly reveals histamine consistent with local mast cell degranulation (5, 50), it may be that targeting mast cells could limit flares by inhibiting recurrent waves of arthritis induction. Furthermore, mast cell numbers expand greatly in chronically inflamed joints, where they are capable of elaborating a range of growth factors, proangiogenic compounds, and other mediators with potentially substantial importance for the course of chronic arthritis (51). Although our results now delineate one discrete contribution of this lineage to inflammatory joint disease, the involvement of mast cells in the pathogenesis of RA is likely to be multifaceted and represents an important topic for further investigation.

Materials and Methods

Animals. C57BL/6J (B6), *Kit*^{w}/*Kit*$^{w\text{-}v}$ (W/W^v), WBB6F1, TNF$^{-/-}$ (52), and BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME). C5aR$^{-/-}$ mice were a gift from Craig Gerard (Children's Hospital Boston) (53). FcεR1$\alpha^{-/-}$ mice on a BALB/c background were kindly provided by Jean-Pierre Kinet (Beth Israel Deaconess Medical Center, Boston, MA) (35). FcR$\gamma^{-/-}$ and FcγRII$^{-/-}$ mice were purchased from Taconic (Germantown, NY) (32, 33). IL-1$^{-/-}$ and K/BxN mice were maintained as described (8, 21). Male mice 6–10 weeks of age were used for all experiments unless specified otherwise. All procedures were approved by the Dana–Farber Cancer Institute Animal Care and Use Committee.

Serum Transfer Protocol, Arthritis and Histologic Scoring, and Cytokine Reconstitution Experiments. Arthritis was induced in recipient mice by transfer of arthritogenic K/BxN serum on days 0 and 2 i.p. as described (8). For histologic scoring, ankle sections were scored in blinded fashion as described (54). For cytokine reconsistution, animals were administered recombinant murine IL-1β or TNF (R&D Systems, Minneapolis, MN)

or vehicle by i.p. injection beginning 4 h after the initial dose of K/BxN serum.

BMMC Culture, Mast Cell Engraftment, and Assessment of Serum and Splenic IL-1β Protein and mRNA. BMMC were generated and engrafted as described (14). K/BxN serum (150 μl $\times$ 1), IL-1β (2.5 μg), LPS (50 μg; *Salmonella enterica* serotype abortus equi) (Sigma, St. Louis, MO) or mock treatment were followed 4.5 h later by blood and tissue harvest. Tissue lysates were prepared in cold lysis buffer (10 mM Tris, pH 7.4/150 mM NaCl/1% Triton X-100/0.05% SDS) containing PMSF (Sigma). IL-1β was assessed via ELISA (R&D Systems) and protein via Bradford assay. Tissue for RNA was snap-frozen, mechanically dispersed in RNase-inhibiting buffer, and lysed by using QiaShredder columns (Qiagen, Valencia, CA). RNA was extracted by using RNeasy (Qiagen). Quantitative RT-PCR was performed as described (55) and normalized to splenic RNA from a BALB/c mouse treated with 50 μg of LPS i.p. 6 h before being killed.

Splenectomy and *in Vitro* Stimulation of BMMC. Splenectomies were performed as described (56). Animals were allowed 2–3 weeks to recover from surgery before the initiation of arthritis. Cultured BMMC were preincubated overnight at 37°C in the anti-FcγRII/III antibody 2.4G2 or isotype control (BD Pharmingen, San Diego, CA), washed, and resuspended at 5×10^5 cells per round-bottom well with cross-linking donkey anti-rat F(ab′)2 fragments (The Jackson Laboratory). After 6 h of incubation at 37°C, plates were gently centrifuged and supernatants were harvested for β-hexosaminidase determination as described (30). Cell pellets were resuspended in cold lysis buffer. IL-1α (BD Pharmingen) and IL-1β (R&D Systems) were determined by ELISA.

Statistical Analysis. Differences were considered significant at $P < 0.05$ by Student's *t* test. To compare curves, the area under the curve was calculated for each animal in an experimental set followed by Student's *t* test between groups (Prism 3.03; GraphPad Software, San Diego, CA).

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