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Essential Role of Neutrophils in the Initiation and Progression of a Murine Model of Rheumatoid Arthritis

Brian T. Wipke and Paul M. Allen

Rheumatoid arthritis (RA) in humans is a debilitating and chronic autoimmune disease characterized by chronic inflammation of the distal joints. Affected joints display hyperplasia of the synovia with increased synovial fluid volume, large cellular infiltrates of several cell types (neutrophils, macrophages, fibroblasts, T cells, and dendritic cells) in the synovial and periarticular regions, complement deposition, high levels of proinflammatory cytokine expression, and eventual erosion and remodeling of the cartilage and bone of the joint (reviewed in Refs. 1 and 2). However, it has been difficult to study the initial stages of disease because afflicted individuals are normally diagnosed after the onset of severe and chronic joint inflammation. Small animal models of arthritis in which disease induction is synchronized and predictable allow investigators to determine the contributions of specific cell types and effector molecules to the multiple steps of disease induction and pathogenesis.

The most extensively used small animal model of RA is type II collagen-induced arthritis (CIA) (3–7), in which immunization of mice with heterologous type II collagen in adjuvant induces a cross-reactive immune response to murine type II collagen. This autoimmune reaction is mediated by immune complex formation and shares many characteristics with human RA (reviewed in Ref. 8). Synovial inflammation (synovitis) can be induced with single mAbs specific for type II collagen (9), and mixtures of type II collagen-specific mAbs against specific regions of collagen cannot only induce synovitis, but also cause clinical arthritis (10), indicating that anti-collagen Ab deposition in the joints triggers the complete range of arthritic symptoms. The CIA model has also been useful for studies of cytokine expression in the synovium and synovial fluid compartments (8), determination of the relative roles of complement (6) and Ig Fc receptors in disease initiation and pathogenesis (reviewed in Ref. 11), and development of effective anti-inflammatory therapeutics such as neutralizing Abs to IL-1 (12, 13) and TNF-α (14–16).

Recently, a spontaneous murine disease with most of the characteristics of human RA was fortuitously discovered by breeding the KRN transgenic TCR, specific for bovine RNase (42–56)/I-Akd, mouse to the nonobese diabetic (NOD) background (17). The F1 generation between KRN and NOD (abbreviated K/B×N) spontaneously develops a progressive joint-specific autoimmune disease between 3 and 5 wk of age, characterized by rapid symmetrical onset of peripheral joint inflammation that is restricted primarily to the joints of the front and rear limbs. Pathology of disease in K/B×N mice is similar to human RA, with pannus formation, synovial hyperplasia, increased synovial fluid volume, cellular infiltrates, and chaotic remodeling of cartilage and bone in the distal joints in later stages. The K/B×N mouse model also exhibits elevated expression of proinflammatory cytokines, hypergammaglobulinemia, and autoreactive Ab production (18), all of which can be found in human RA patients. One difference between the two diseases is the absence of detectable rheumatoid factor in K/B×N mice (17), although ~20–30% of human RA patients are also negative for serum rheumatoid factor (19). Immune complexes of rheumatoid factor-IgG and potentially other Ab-Ag immune complexes can be found in the joints of human RA patients (20), but their role in joint pathology and disease progression has yet to be ascertained.

In the K/B×N model, KRN TCR transgenic cells recognize a mouse (self)-derived peptide bound to I-Akd7, presented by B cells and other MHC class II-positive APCs (18). The autoantigen for...
both K/B×N arthritogenic Ig and KRN T cells was recently identified (21) as glucose-6-phosphate isomerase (GPI), an ubiquitous cytoplasmic enzyme that catalyzes the interconversion of fructose-6-phosphate and glucose-6-phosphate during glycolysis. Our laboratory has recently established the molecular basis for the dual ability of the KRN TCR to recognize RNase (42–56)/I-A<sup>D</sup> and GPI (282–294)/I-A<sup>D</sup> (22, 23). A working model of K/B×N disease initiation postulates that GPI-specific B cells endocytose GPI via surface Ig receptors and present the I-A<sup>D</sup>-restricted epitope to incompletely tolerized CD4<sup>+</sup> KRN T cells, which in turn provide specific help in maturation and Ig isotype switching, leading to autoantibody production (17, 18, 21) and subsequent induction of joint inflammation.

Transfer of serum or purified Ig from arthritic K/B×N mice induces a synchronized joint-specific inflammatory reaction that mimics the K/B×N disease (18), indicating that arthritogenic Ig can induce synovitis and rheumatoid-like arthritic disease. The disease induced by a single administration of serum eventually resolves, unless arthritogenic Ig is repeatedly transferred into recipients (18). The development of an easily inducible model of RA with a rapid, synchronized onset facilitates the study of the pathogenic mechanisms involved in the initiation of joint autoimmunity.

In this study, we found that neutrophils play an indispensable role in disease initiation in the K/B×N serum transfer model of RA. We used a mAb to deplete neutrophils in recipient mice and found that this treatment completely blocked the acute joint-specific inflammatory response normally induced by K/B×N serum transfer, and caused rapid and profound reversal of joint inflammation in diseased mice. Furthermore, we determined that neither the inducible NO synthase (iNOS) nor gp91<sup>phox</sup> gene products are required for the serum-induced joint inflammation.

Materials and Methods

Mice

NOD mice (6–8 wk old) were obtained from Taconic Farms (Hudson, NY). KRN TCR transgenic mice on a B6 background (K/B, the generous gift of D. Mathis and C. Benoist, Harvard Medical School, Boston, MA) were bred to NOD mice to generate K/B×N mice. Mice deficient for iNOS (24) or gp91<sup>phox</sup> (25) gene expression, C57BL/6J, B6.AKR (H-<sup>2</sup> K<sup>B</sup>), B6.AKR-RAG1<sup>−/−</sup> mice were obtained from The Jackson Laboratory (Bar Harbor, ME), and bred and housed under specific pathogen-free conditions in the animal colony at Washington University (St. Louis, MO). All mice were sex matched and age matched for experiments, and were between 6 and 10 wk of age.

Arthritic serum

Serum was separated from blood obtained from arthritic K/B×N mice (6–12 wk old) and frozen at −70°C. To prepare larger pools for in vivo experiments, frozen serum samples were thawed, centrifuged at 12,000 rpm for 10 min, pooled and sterile filtered, and frozen in aliquots at −70°C. Before injection, serum aliquots were thawed, centrifuged, and diluted with PBS. Each batch of pooled serum was titrated in B6.AKR mice (groups of 3–5 mice per dose) for its ability to transfer joint-specific disease and to determine an effective dose of serum for subsequent serum transfer experiments. A dose of 250 μl per mouse was selected, as it consistently gave disease induction in 100% of the mice.

Antibodies

The rat IgG2b mAb RB6-8C5 (26) was purified using protein G-Sepharose 4 Fast Flow affinity matrix (Pharmacia, Piscataway, NJ) from ascites produced in SCID mice. GK1.5 mAb (27) against mouse CD4 (rat IgG2b) was purified from ascites by saturated ammonium sulfate precipitation (45% saturated). Antibodies were collected using a FACS Calibur and analyzed using CellQuest software (BD Biosciences, Mountain View, CA). Gates and histograms were set using untreated B6.AKR peripheral blood stained with RB6-8C5 or GK1.5.

K/B×N serum transfer model

Serum as prepared above was injected i.p., and clinical scores were determined daily based upon evidence of redness and swelling, using a scale ranging from 0 to 4, as described previously (17). Physical measurement of ankle thickness was performed using a Fowler Metric Pocket Thickness Gauge (Ralmikes Tool-A-Rama, Middletown, NJ). Ankle measurements were made above the footpad, axially across the ankle joint, and rounded off to the nearest 0.05 mm. Data are presented as the mean of individual ankle thicknesses within a group of mice (3–6 mice per group). Mean percent inhibition of ankle swelling due to RB6-8C5 treatment was calculated using the following formula: 1 − (experimental mean ankle thickness − PBS baseline mean ankle thickness)/(PBS mean ankle thickness − PBS baseline mean ankle thickness). Student t tests for paired data were performed using Kaleidograph software (Synergy Software, Reading, PA) on calculated values from data of three identical experiments, with a total of 12 mice per experimental condition.

Histology

Tissue samples were prepared by fixing tissues 24–48 h in 10% phosphate-buffered formalin (J.T. Baker, Phillipsburg, NJ). NOD and KRN TCR transgenic (K/B×N) or control (C57BL/6J) mice were de-identified by treatment with Decal Overnight Bone Decalcifier solution (Decal Chemical, Congers, NY) for 2 days with gentle rocking and daily replacement of the Decal solution. Samples were then washed with PBS, dehydrated with a series of ethanol washes (50% ethanol, followed by 70% ethanol), and embedded in paraffin. Sections of tissue 4 μm thick were stained with H&E. Representative sections from individual mice within groups of up to six mice were selected to illustrate the general state of each group’s ankle joints.

Results

Polymorphonuclear neutrophils were found in periarticular and synovial regions of ankle joints within 48 h of serum transfer

To determine the timing of inflammatory cell recruitment to affected joints following K/B×N serum transfer, we performed histological studies on ankle joints over time. Injection of serum reproducibly resulted in rapid bilateral ankle swelling (Fig. 1A) and increased clinical index scores (Fig. 1B) in B6.AKR mice within 24–48 h post transfer. Ankles continued to increase in size up to 7 days post transfer.

Sagittal sections of hind ankles through the center of the joint 12 h after serum transfer showed no change in the size or condition of the ankle and metatarsal joints compared with control mice (data not shown). Twenty-four hours after serum transfer, the ankle joint (Fig. 1A and B) and wrist joint (data not shown) were inflamed, with redness (erythema), tenderness, and measurable ankle swelling. Histological examination at 24 h (Fig. 1D, ×50 magnification) revealed a slight increase in the size of the articular
space contained within the synovium, as evidenced by a larger gap between the talus and the synovial membrane, consistent with observed edema in the region. The synovial fluid space was clear and free of cellular infiltrate, and the synovial membrane appeared normal (data not shown). Thirty-six to forty hours after serum transfer, ankle joints continued to increase in thickness and clinical index severity, with nearly all of the extremities involved (Fig. 1, A and B). Kinetics of joint inflammatory disease caused by transfer of K/B×N serum. A representative group of mice (n = 3) received a single injection of 250 μl K/B×N serum i.p., and were monitored for changes in ankle thickness and clinical index score. Detectable swelling and inflammation occur within 48 h. Results shown are from one experiment and are representative of more than five experiments. C–H, Histological time course of cellular infiltration into periarticular and intraarticular spaces following serum transfer. Mice received a single injection of 250 μl K/B×N serum i.p., and ankle joints were harvested at 12- to 24-h increments, treated for histology as detailed in Materials and Methods, and stained with H&E. Shown are ankle cross-sections of a normal B6.AKR mouse (C), and serum recipient mice at 24 h (D), 40 h (E and F), and 48 h (G and H), which are representative of the general inflammatory response in multiple mice for each time point. Photographs of representative ankle joint sections are shown at magnifications of ×50 (C–E) and ×500 (F–H).

Neutrophil depletion in vivo dramatically blocked the ability of K/B×N arthritogenic serum to induce joint-specific inflammatory reactions in serum transfer recipients

To further investigate the role of neutrophils in the serum transfer model, we used a depleting mAb (RB6-8C5) specific for a neutrophil-restricted surface marker (Ly-6G), which has been used in numerous unrelated studies to deplete neutrophils in vivo (31–37). B6.AKR mice were treated with purified RB6-8C5 rat mAb or vehicle alone (PBS) on days −2, 2, and 5 by i.p. injection (250 μg per injection), and K/B×N serum was injected i.p. on day 0. Clinical score and ankle thickness were monitored for 7 days after serum transfer, and the efficiency of neutrophil depletion in peripheral blood was monitored by flow cytometry. Data from two such RB6-8C5 depletion experiments and their associated FACS monitoring of peripheral blood are shown in Fig. 2. In the first experiment (Fig. 2, A–C), B6.AKR mice were treated with either three doses of RB6-8C5, PBS, or the isotype-matched CD4-depleting rat Ab GK1.5 beginning 1 day before serum transfer (day
Groups of five mice were treated with either PBS (0.5 ml), RB6-8C5 (250 μg each) or GK1.5 mAb (50 μg). Mice treated with RB6-8C5 showed no signs of clinical disease through day 7 post transfer (Fig. 2, A and B). We found that there was no observable difference between groups treated with either PBS or GK1.5 in either the severity of ankle swelling (Fig. 2A) or clinical index scores (Fig. 2B). There also was no difference in the time of onset for inflammation, as both groups of mice showed measurable swelling at 2 days post transfer and peaked on days 6–7. The extremities of the PBS- and GK1.5-treated groups became reddened and inflamed within 48 h, while the ankles and wrists of RB6-8C5-treated mice showed no signs of inflammatory response and were indistinguishable from those of control mice that did not receive serum.

Depletion of RB6-8C5-positive peripheral blood leukocytes was essentially complete 1 day after treatment and remained extremely low (<1% of total PBL) throughout the experiment (Fig. 2C). Additionally, analysis of FACS plots of forward light scatter vs side light scatter demonstrated the near-total absence of cells in the neutrophil region of the plot, which in normal mice is >95% positive for RB6-8C5 staining (data not shown). CD4+ T cells were completely absent from the peripheral blood of GK1.5-treated mice by FACS analysis (data not shown), yet the progression and severity of serum-transferred disease were unaffected, showing that the protective effect was specific for the depletion of neutrophils.

In addition to its effect on neutrophils, RB6-8C5 mAb treatment has been reported to lead to a slow reduction in the number of CD8+ T cells over the course of several days (36, 38, 40). To ensure that the protective effect of RB6-8C5 mAb treatment was not due to depletion of CD8+ T cells, serum transfer disease was evaluated in RAG1-deficient mice (RAG1−/−), which lack B and T cells. There was no significant difference between RAG1-deficient B6.AKR and wild-type B6.AKR mice in relation to the onset of inflammation, progression, or severity of disease following serum transfer, demonstrating that T and B lymphocytes were dispensable in the serum transfer model. Furthermore, RB6-8C5 mAb treatment protected RAG1-deficient mice from disease induction (Fig. 2, D and E). Collectively, the results demonstrated that K/B×N Ig were unable to induce this joint-specific reaction in recipient mice in the absence of a normal neutrophil compartment. This indicates that RB6-8C5-positive neutrophils play an important role in initiating this Ab-mediated disease.

**Protection against K/B×N serum-induced joint swelling by RB6-8C5 neutrophil depletion correlates with normal ankle morphology and absence of cellular infiltrates**

The ankles of RB6-8C5-treated mice showed remarkably normal morphology at 5 and 7 days post serum transfer, virtually indistinguishable from that of a normal mouse ankle. Ankles from RB6-8C5-treated mice 5 days after serum transfer showed no signs of inflammatory infiltrate or synovial hyperplasia (Fig. 3, B and C). In contrast, ankles of PBS-treated mice demonstrated massive inflammatory infiltrate, increased synovial fluid volume, and synovial hyperplasia with large numbers of neutrophils present in the synovial fluid (Fig. 3, A and B). The lack of detectable infiltration and synovial hypertrophy in these histological studies of neutrophil-depleted mice correlates with the absence of measurable swelling (Fig. 2A) or visible inflammation (Fig. 2B), indicating that the presence and infiltration of neutrophils are obligatory for the characteristic changes induced by transfer of K/B×N serum. Therefore, neutrophils must possess properties or functions that are important or even indispensable for the inflammatory response triggered by K/B×N Ig transfer.

**Testing of mice deficient for generation of reactive nitrogen species and reactive oxygen species in the K/B×N serum transfer model**

To begin to determine the mechanism(s) by which neutrophils may act to induce joint disease, we assessed the responses of mice deficient for iNOS2 (iNOS2 knockout mice), which are unable to generate NO (24), and gp91phox-deficient mice that are unable to generate hydrogen peroxide by the NADPH-dependent pathway.
relative to B6 and $\text{gp91 phox}$ deficient mice and $i\text{NOS2}$ observed slightly enhanced inflammation in similar kinetics (Fig. 4, A–D). Results from one of three experiments are shown in Fig. 4, in which we found that relative to congenic B6 mice, $\text{gp91 phox}$-deficient mice and $i\text{NOS2}$ knockout mice developed arthritis with similar kinetics (Fig. 4, A and B). In two of three experiments, we observed slightly enhanced inflammation in $i\text{NOS2}$ knockout mice relative to B6 and $\text{gp91 phox}$-deficient mice. Neither strain proved to be more resistant than B6 mice to the inflammation induced by $\text{K/B3N}$ serum transfer. Histological analysis of ankle joints from these strains of mice failed to reveal any gross differences in joint morphology (data not shown), suggesting that neither reactive intermediate pathway is critical for the neutrophil-dependent inflammatory response to $\text{K/B3N}$ serum.

Reversal of $\text{K/B3N}$ serum transfer inflammation with neutrophil depletion via $\text{RB6-8C5}$ treatment

The depletion of neutrophils before serum transfer prevents disease. It was next important to determine whether neutrophil depletion could affect disease progression after the transfer of serum. Groups of B6.AKR mice were treated with a single i.p. injection of 250 μg $\text{RB6-8C5}$ on days −1, 0, 1, 2, or 3 days after $\text{K/B3N}$ serum was transferred. This protocol differed from our previous ones, in that we were only administering a single dose of $\text{RB6-8C5}$.

A single injection of $\text{RB6-8C5}$ was equally effective in completely preventing joint inflammation for ~6 days, regardless of whether it was administered 1 day before serum transfer (day −1) or at the same time as serum (day 0, Fig. 5, B and C). At 1 day after serum transfer, the remaining untreated groups of mice demonstrated visible signs of inflammation similar to mice in Figs. 1 and 2 (data not shown) and only slightly elevated ankle measurements (Fig. 5D–G). Treatment with $\text{RB6-8C5}$ beginning 1 day after serum transfer (day +1) immediately and effectively blocked inflammation and clinical manifestations of the disease within 24 h of treatment (Fig. 5D). Treatment with $\text{RB6-8C5}$ 2 and 3 days after serum transfer quickly reversed the joint-specific inflammatory reaction, as measured by ankle swelling and clinical scores. Mice that received $\text{RB6-8C5}$ on day 4 (G) and day 5 (H) also showed a rapid decline in ankle thickness beginning ~1 day after treatment, indicating that at this time the inflammation is also reversible or at least can be ameliorated by neutrophil depletion. All of the treated groups showed significant benefit from neutrophil depletion, as determined by reduction in erythema, puffiness, and joint thickness (data not shown). Inhibition of ankle swelling by neutrophil depletion became less effective when initiated after day 3 of serum transfer (Fig. 5I). Mice were analyzed for the extent of neutrophil depletion on days 0, 2, 4, and 7 by FACS analysis. By day 5–6 after $\text{RB6-8C5}$ mAb injection, neutrophils began to reappear in peripheral blood due to clearance of Ab from the bloodstream and tissues and maturation and release of neutrophils from the bone marrow. There is a clear correlation between the time of recovery of $\text{RB6-8C5}$+$\text{ peripheral neutrophils and the onset or resumption of joint inflammation (data not shown). These studies revealed that the early stages of experimental immune complex-induced arthritis, up to day 3, could be completely reversed by depletion of neutrophils, and disease severity was ameliorated up through day 5. These experiments indicate that not only are neutrophils critically involved during the first 3–5 days of the disease, but they are also required for the continuation and progression of the immune complex-mediated inflammatory state.

Discussion

Transfer of $\text{K/B3N}$ serum Ig into recipient mice induces the synchronized and rapid onset of joint inflammation. We used this model to determine the importance of neutrophils in the initiation,
progression, and maintenance of inflammatory joint disease in this experimental mouse model of rheumatoid-like polyarthritis. We found that Ab-induced neutrophil depletion caused a complete block in the ability of K/B×N serum to induce joint swelling in recipient mice, and this protection from inflammation could be maintained for periods of at least 7 days with repeated RB6-8C5 Ab treatment. The foot and ankle joint spaces remained free of infiltrating neutrophils by histology, and the synovia of the ankle remained free of cellular infiltrate and were of normal size and morphology. When the neutrophil compartment was allowed to recover by termination of RB6-8C5 treatment, the repopulation of the peripheral blood neutrophil population (by maturation from immature precursor cells) correlated closely with the observed onset of joint inflammation, indicating an active role for neutrophils in the early stages of this disease rather than as late arrivals to well-established sites of inflammation. Joint inflammation could be completely blocked or reversed by neutrophil depletion if initiated by 3 days postserum transfer, but became progressively less effective when initiated at later time points, indicating the probable involvement of other cell types at later stages in the K/B×N serum transfer model. Formation of scar tissue and fibroblast proliferation may also be contributing factors to residual ankle thickening after prolonged inflammation.

This model disease can be transferred to normal mice with purified GPI-specific IgG from arthritic mice (21), strongly suggesting that the initial triggering event is the formation and/or deposition of GPI-IgG immune complexes in the joint spaces. Therefore, it is likely to share features with other immune complex-triggered autoimmune models (11, 41, 42). Local mononuclear cells and possibly mast cells (43) become activated by immune complex-activated complement fragments (44, 45), tissue damage, and/or FcγR cross-linking (11, 46), and release proinflammatory cytokines such as IL-1β and TNF-α (43, 47) in or near the affected tissue (e.g., joints), which induces some relatively low level of neutrophil recruitment. These few initial neutrophils become activated, extravasate, and home to the joints, where they proceed to help create a proinflammatory cytokine milieu that is necessary to maintain and expand the joint inflammatory response. In a rat model of arthritis unrelated to the K/B×N model, repeated injection of streptococcal cell wall extract causes joint inflammation, which has also been shown to be neutrophil dependent and requires P-selectin, ICAM-1, and macrophage-inflammatory protein-2 (48), suggesting these molecules are likely to be important.
in the K/B×N model for neutrophil recruitment. In the CIA model, it has been clearly demonstrated that autoreactive Abs to type II collagen initiate inflammation by binding to articular cartilage and causing activation of complement, C3 deposition (44), and eventual cleavage of C5 (45). Although the K/B×N RA model is also autoantibody mediated and shares pathology with CIA, the Ag (GPI) is not joint specific (21), and deposition of GPI-autoantibody immune complexes in the joints of mice has yet to be demonstrated, and remains an intriguing question.

Potential neutrophil effector mechanisms that may be critical for the induction and progression of joint inflammation in the murine K/B×N serum transfer model include release of granules containing degradative enzymes, and the production and release of proinflammatory cytokines. Neutrophil granule contents such as myeloperoxidase, elastase, matrix metalloproteinases, and collagenase (41, 49) can cause further damage to the tissue and amplify the neutrophil response, which has been observed in an Ab-mediated model of bullous pemphigoid (50). Alternatively, activated neutrophils are also capable of releasing proinflammatory cytokines such as TNF-α, IL-1, IL-6, and TGFβ (51, 52), potentially affecting the activities of both neutrophils and other cell types, such as resident mononuclear cells and chondrocytes. TNF-α is at the apex of a proposed cascade model of proinflammatory cytokines in rheumatoid synovial tissue (2), and the role of TNF-α as a dominant proarthritogenic cytokine has been demonstrated with mice transgenic for human TNF-α (53). Neutrophils may also interfere with the balance of IL-1 and endogenous IL-1 receptor antagonist (IL-1ra) activity in the joint (54), either by direct secretion of IL-1 or through TNF-α-mediated IL-1 induction. Mice that are deficient in the endogenous IL-1ra develop a chronic, progressive joint inflammation in several model systems of immunopathologic disease, indicating the importance of a balance between IL-1 and IL-1ra in normal joint physiology and homeostasis of inflammatory cytokines.

Finally, TGFβ is another proinflammatory cytokine found in synovial tissue and fluid (55) and is a powerful neutrophil chemomtactant (56). As with TNF-α and IL-1, neutrophils can both produce and respond to TGFβ, providing a potential amplification mechanism for continued neutrophil activation and recruitment. In combination with their capacity to make TNF-α and IL-1, one can propose a model for K/B×N serum transfer in which initial mononuclear cell activation and cytokine release in the joint tissues induces some level of initial neutrophil recruitment. The initial wave of recruited neutrophils would then be largely responsible for amplifying and sustaining recruitment of neutrophils to the inflamed tissues through the continued generation and release of proinflammatory cytokines such as TNF-α, IL-1, and TGFβ.

In contrast to cytokine secretion and degranulation of proenzymes that are usually directed toward extracellular targets, the oxidative species produced by the iNOS pathway (e.g., NO) and the NADPH oxidase pathway (hydrogen peroxide) are usually directed intracellularly against phagocytosed bacteria or particles contained within a phagosome. However, it has recently been proposed (49) that adherent neutrophils could release such reactive species into small pockets of synovial fluid, leading to inappropriate activation of proenzymes such as elastase or metallothionein proteases, and damage of the synovial fluid hyaluronan or cartilage. In several model systems of immunopathologic disease, inhibition of either NADPH or iNOS pathways or both together has yielded a reduction in the severity of inflammation or disease (29, 57, 58). Unexpectedly, we found that mice deficient for either gpr150 or iNOS2 activity presented similar disease phenotypes as wild-type control mice. There was no significant reduction in the severity of disease induced by K/B×N serum transfer, nor was there any significant alteration in the time of disease onset for these mutant strains of mice. Interestingly, iNOS2 knockout mice demonstrated enhanced swelling relative to B6 mice in two of three experiments. This suggests that neither of these two pathways are obligatory for immune complex-triggered joint inflammation in the K/B×N serum transfer model, although one might expect that they are likely to be involved in tissue damage once a joint becomes inflamed. Indeed, other studies have shown that overproduction of NO and its reaction product, peroxynitrite, contributes to the pathophysiology of RA and joint inflammation (59, 60).

To summarize, it has been hypothesized, but not directly shown, that neutrophils are an important component of inflammatory responses to immune complex deposition in the joints because they are found there in high numbers. This study convincingly demonstrates that neutrophils are an essential component of the K/B×N autoreactive IgG transfer model of rheumatoid-like arthritis, and that an induced state of neutropenia confers protection from joint-specific inflammation. These results are important because they demonstrate for the first time that neutrophils play an essential inductive role in the generation of joint-specific inflammation in the K/B×N serum transfer model (e.g., one or more of the properties of neutrophils are responsible for early stages of inflammation in the K/B×N). The mechanism by which neutrophils are recruited specifically to the joint in the K/B×N model, and which neutrophil characteristics are important in this location, are questions that bear further consideration and will be the subject of future experiments. Further studies to elucidate the exact operative mechanisms exerted by neutrophils may point to potential approaches for pharmacological intervention in other inflammatory diseases such as human RA, to help ameliorate or limit the severity and scope of joint disease.

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