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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

Neutrophils are prominent participants in the joint inflammation of human rheumatoid arthritis (RA) patients, but the extent of their role in the inductive phase of joint inflammation is unknown. In the K/B×N mouse RA model, transfer of autoreactive Ig from the K/B×N mouse into mice induces a rapid and profound joint-specific inflammatory response reminiscent of human RA. We observed that after K/B×N serum transfer, the earliest clinical signs of inflammation in the ankle joint correlated with the presence of neutrophils in the synovial regions of recipient mouse ankle joints. In this study, we investigated the role of neutrophils in the early inflammatory response to transferred arthritogenic serum from the K/B×N transgenic mouse. Mice were treated with a neutrophil-depleting mAb before and following transfer of arthritogenic serum and scored for clinical indications of inflammation and severity of swelling in ankle joints and front paws. In the absence of neutrophils, mice were completely resistant to the inflammatory effects of K/B×N serum. Importantly, depletion of neutrophils in diseased recipient mice up to 5 days after serum transfer reversed the inflammatory reaction in the joints. Transfer of serum into mice deficient in the generation of nitrogen or oxygen radicals (inducible NO synthase 2 or gp91phox genes, respectively) gave normal inflammatory responses, indicating that neither pathway is essential for disease induction. These studies have identified a critical role for neutrophils in initiating and maintaining inflammatory processes in the joint.

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 RNPase (42–56)/I-A^d and GPI (282–294)/I-A^E (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^E-restricted epitope to incompletely tolerized CD4^+ 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. 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^2) nor gp91^phox 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 (German- town, 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^phox (25) gene expression, C57BL/6J, B6- AKR (H- 2^b), B6- AKR- RAG1^-/- 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 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% final) and dialysis against PBS, pH 7.4, before being stored at −70°C.

Ab depletion in vivo

For in vivo neutrophil depletion, 250 μg RB6-8C5 mAb was diluted to 0.5 ml with PBS and injected i.p. at 3-day intervals beginning 1 day before serum transfer, except for the experiment described in Fig. 4. This dose of Ab is equal to or greater than has been shown previously to be efficacious in completely eliminating neutrophils in vivo (28–30). We determined in early experiments that a single injection of RB6-8C5 is not sufficient to completely block joint inflammation for more than ~5–6 days, as demonstrated by the appearance of clinical signs of inflammation and measurable thickening of the ankle joints. We attribute this to clearance of the RB6-8C5 Ab from the bloodstream and tissues and the maturation and release of new neutrophils from the bone marrow. Previous studies of peripheral blood cell types in RB6-8C5-treated mice have established that macrophages, NK, and T and B lymphocyte populations are not significantly affected by such methods (31–37). Several studies have demonstrated that RB6-8C5 Ab fails to bind to mature macrophages, monocytes, or splenic B and T lymphocytes by either flow cytometry analysis (32, 36) or immunofluorescent microscopy (33). CD^8^ T cells have been previously reported to be moderately affected by RB6-8C5 treatment (36, 38, 39), but the effect on CD^8^ cells occurs several days after neutrophils are depleted (38), and would be of no consequence in this model, as T cell-deficient RAG1 mice are equally susceptible to disease (see Fig. 2, D and E). For the control anti-CD4 Ab, 50 μl ammonium sulfate-precipitated GK1.5 mAb was diluted to 0.5 ml with PBS and injected i.p. Depletion was monitored by flow cytometry using biotinylated RB6-8C5 and GK1.5 (PharMingen, San Diego, CA) and streptavidin-R-PE (Caltag, Burlingame, CA) using standard procedures. A minimum of 20,000 total events was collected using a FACSCalibur 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, Middlesex, 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 depletion was calculated using the following formula: 1 − (experimental mean ankle thickness − experimental 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). Tissues were 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 five to six were selected to illustrate the general state of each group’s ankle joints.

Results

Polyomarwhocellular 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. 1, A 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). Histological analysis at 48 h showed an increased synovial fluid volume and the presence of neutrophils in the synovial fluid surrounding the talus, calcaneus, and distal tibia/fibula (Fig. 1, E, ×50 magnification, and F, ×500 magnification). Neutrophils also began to accumulate in the loose connective tissue posterior to the ankle joint between the Achilles tendon and the talus, and also in the tissues surrounding the synovium on the lateral and anterior portions of the ankle joint.

At 48 h, all of the mice showed significant outward signs of ankle joint inflammation and swelling, with mean ankle sizes increased ~20% in diameter (Fig. 1A). Histological analysis at 48 h showed initial signs of synovial membrane expansion surrounding the articular spaces of the joint, with neutrophils and other infiltrating cells in close proximity to the joint spaces and in the synovial fluid (Fig. 1, G and H, ×500 magnification). Neutrophil numbers were greatly increased, infiltrating the low density connective tissue found posterior to the ankle joints, and also on the sides of the ankle joint just below the malleoli of the tibia and fibula (data not shown).

**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, 3, 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). FIGURE 2. Depletion of neutrophils by administration of RB6-8C5 Ab renders mice resistant to K/B×N serum-induced joint inflammation. Data from two similar experiments are shown in A–C and D–F, respectively. Groups of five mice were treated with either PBS (0.5 ml), RB6-8C5 (250 μg each) or GK1.5 mAb (50 μl purified ascites each, A–C only) by injection i.p. on days −1, 2, and 5 as indicated by arrows on the x-axis. On day 0, each mouse received 250 μl K/B×N serum i.p. A and D, Depletion of neutrophils, but not CD4+ T cells prevents joint inflammation. Mean ankle thicknesses were determined daily for each group of mice by measuring the width of ankles across the medial-lateral axis of the joint. SDs for mean ankle thickness were determined daily for each group of mice by Ab staining and flow cytometry, as described in Materials and Methods. Data are presented as the mean percentage of total PBL staining 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 postserum 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, C and D). 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 deficient mice and observed slightly enhanced inflammation in iNOS2 similar kinetics (Fig. 4, A)

Reversal of K/B×N serum transfer inflammation with neutrophil depletion via 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 RB6-8C5 on days −1, 0, 1, 2, or 3 days after K/B×N serum was transferred. This protocol differed from our previous ones, in that we were only administering a single dose of RB6-8C5.

A single injection of 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. 5,D–G). Treatment with 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 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 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 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 RB6-8C5 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 K/B×N 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 proarthritic 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 on certain mouse backgrounds, illustrating 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 chemotactant (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 gp91phox 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 pharmacologic 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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