Lack of Requirement of Osteopontin for Inflammation, Bone Erosion, and Cartilage Damage in the K/BxN Model of Autoantibody-Mediated Arthritis

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Objective. **Osteopontin (OPN) is a secreted glycoprotein involved in a range of physiologic processes, including inflammation, immunity mediated by Th1 cells, and bone remodeling. It is expressed in the joints of rheumatoid arthritis patients and has been the subject of conflicting reports concerning its role in arthritis induced by antibodies against type II collagen. This study assessed the role of OPN in the K/BxN serum-transfer model of autoantibody-induced arthritis.**

Methods. **Expression of OPN gene transcripts was assessed by microarray analysis of ankle RNA taken at 6 time points after transfer of K/BxN serum. OPN-sufficient or OPN-deficient littermates backcrossed for 10 generations onto the C57BL/6 genetic background were given K/BxN serum. Arthritis severity was measured by ankle thickening and a clinical index. Hind limb sections were stained with hematoxylin and eosin or toluidine blue and scored for inflammation, cartilage damage, and bone erosion.**

Results. **OPN messenger RNA transcripts progressively increased in ankle joints during the course of K/BxN serum-transferred arthritis. OPN-deficient mice receiving K/BxN serum developed arthritis with kinetics and clinical severity comparable with those of OPN-sufficient littermates. Histologic assessment of arthritic joints from OPN-deficient mice revealed synovial hyperplasia, pannus formation, mononuclear cell infiltration, bone erosion, cartilage damage at sites adjacent to and distal from pannus invasion, and tartrate-resistant acid phosphatase–positive multinucleated cells at sites of bone erosion. Histopathologic scoring demonstrated comparable levels of inflammation, cartilage damage, and bone erosion in OPN-sufficient and OPN-deficient mice.**

Conclusion. **OPN does not have a required role in inflammation, bone erosion, and cartilage damage in the K/BxN serum-transfer model.**

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Rheumatoid arthritis (RA), a chronic inflammatory disease involving synovial joints, is characterized by synovial hyperplasia, leukocyte infiltration into synovium, focal bone erosion, and formation of a region of invasive synovial tissue, or pannus, that destroys joint structures. One candidate regulator of inflammation and bone destruction in RA is osteopontin (OPN), which is also known as early T lymphocyte activation gene 1 (Eta-1).

OPN is a phosphorylated glycoprotein that is present as a cytokine in body fluids and as an immobilized component of the extracellular matrix of mineral-

ized tissues (1–3). It is secreted by many cell types, including T cells, macrophages, osteoblasts, and osteoclasts, and it can be induced by inflammatory mediators such as interleukin-1, tumor necrosis factor α, and platelet-derived growth factor. OPN interacts with integrin receptors and CD44 to induce chemotaxis, promote cell adhesion, and modulate cell function. At sites of inflammation, it promotes macrophage infiltration and dendritic cell migration to lymph nodes (4,5). It is an important mediator of Th1 immunity and protective granuloma responses, and can enhance B cell proliferation and antibody secretion (6–9). OPN has been directly linked to autoimmunity in studies finding that OPN-deficient mice have attenuated experimental autoimmune encephalomyelitis (EAE) (10,11). However, these results have recently been challenged by a group of investigators who observed no attenuation of EAE in more genetically homogeneous OPN-deficient mice (12).

OPN may also play a role in bone resorption. OPN-deficient mice have an increased volume of trabecular bone and reduced bone resorption induced by experimental stresses, including ovariectomy and mechanical stress (13–17). Ectopically transplanted bone from OPN-deficient mice is resorbed less effectively than is transplanted bone from wild-type mice (18). Cultured bone from OPN-deficient mice has less resorption and osteoclast formation than does cultured bone from wild-type mice in response to parathyroid hormone and RANKL/macrophage colony-stimulating factor (19).

A few studies have provided experimental evidence linking OPN to inflammatory arthritis (20). Levels of OPN messenger RNA (mRNA) and protein were found to be elevated in synovial tissue from RA patients compared with synovial tissue from osteoarthritis patients (21,22). In those studies, OPN primarily localized to fibroblasts in the synovial lining and regions of pannus invasion into cartilage. OPN was also detected at sites of osteoclast-mediated bone erosion in mice with collagen-induced arthritis (CIA) and in the cartilage of mice with anti–type II collagen (anti-CII) antibody–induced arthritis (23,24). In a study using the latter system, OPN-deficient mice had reduced levels of inflammation, cartilage damage, and inflammatory cell infiltration compared with OPN-sufficient controls (24). Using the same model, a second group of investigators found that treatment with antibodies against the SLAYGLR epitope of thrombin-cleaved OPN delayed the onset of arthritis and reduced its severity (25). The role of OPN in arthritis remains controversial, however, since an-

other group of investigators found that OPN deficiency did not protect against CIA or anti-CII antibody–induced arthritis (12).

We studied the role of OPN in the K/BxN serum-transfer model of arthritis. In this system, arthritis bearing marked clinical and histologic similarity to RA was induced by intraperitoneal injection of serum from arthritic K/BxN mice, the progeny of KRN T cell receptor (TCR)–transgenic mice (K/B) and nonobese diabetic mice (N) (26,27). Disease is mediated by pathogenic autoantibodies produced against glucose-6-phosphate isomerase, a ubiquitously expressed glycolytic enzyme that deposits on joint surfaces (28,29). We found that although OPN was up-regulated during K/BxN serum-transferred arthritis, it had no required role in this model.

MATERIALS AND METHODS

Mice. OPN-knockout mice were described by Rittling et al (13) and have been backcrossed onto the C57BL/6 (B6) genetic background for 10 generations. Matched littermates from heterozygote crosses or heterozygote/homozygote crosses were used as controls. Genotypes were assessed by genomic polymerase chain reaction and confirmed by OPN enzyme-linked immunosorbent assay (ELISA) of sera from killed animals. K/BxN mice were generated by crossing KRN TCR-transgenic B6 mice with NOD mice (26). These experiments were reviewed by the Harvard Medical School Institutional Animal Care and Use Committee (protocol no. 03024).

Generation of serum-transferred arthritis and clinical scoring. K/BxN serum was collected from 8-week-old arthritic K/BxN mice and pooled for each experiment. Arthritis was induced by intraperitoneal injection of 150 μl of K/BxN serum on days 0 and 2 or on days 0, 2, 7, and 14. In a dose-response experiment, mice were injected with 18.75, 37.5, 75, or 150 μl of K/BxN serum on days 0 and 2. For doses lower than 150 μl, serum was diluted to 150 μl with phosphate buffered saline (PBS). Animals were killed for histologic assessment on day 15 or on days 7, 14, and 21; all remaining animals were killed 21 days after serum transfer.

Two clinical parameters of arthritis, ankle thickness and a clinical index, were measured from the day of injection until the mice were killed. Ankle thickness was measured with calipers (J15; Blet, Lyon, France) and reported as ankle thickening, representing the difference between the ankle thickness on day 0 and at specific time points thereafter. Each limb was scored on a scale of 0 (no observable swelling) to 3 (severe inflammation). The scores for the 4 limbs were added to give the clinical index.

OPN expression analysis. A microarray analysis was performed on ankle RNA extracts taken on days 0, 1, 3, 7, 12, and 18 after serum transfer into B6 mice (Jacobs JP: unpublished observations). Briefly, ankle joints were dissected open, and the exposed synovial tissue was digested in 6M urea/2% sodium dodecyl sulfate. RNA samples prepared from these extracts were amplified using a MessageAmp kit (Ambion,
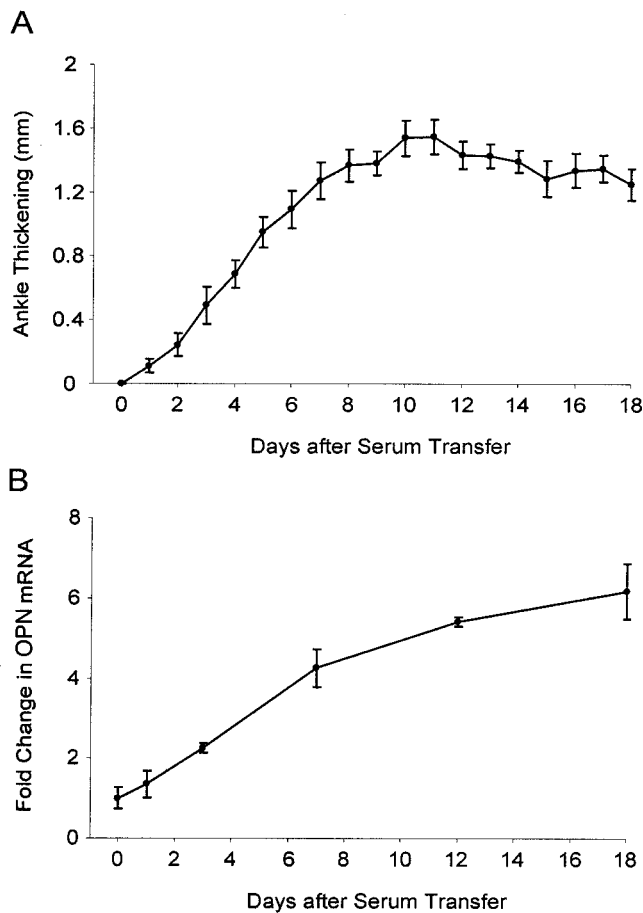
Figure 1. Up-regulation of osteopontin (OPN) mRNA in the ankles of K/BxN serum-transferred mice. A microarray analysis was performed on ankle RNA samples collected on days 0, 1, 3, 7, 12, and 18 after initial injection with K/BxN serum. Each sample consists of pooled ankle RNA extracts from 2–3 mice; 3–6 replicates were performed for each time point. **A,** Time course of arthritis, as represented by the mean ± SEM ankle thickening each day in the 9 mice that were killed on day 18. **B,** Fold change in OPN mRNA expression from day 0 to day 18, as measured by Affymetrix U74Av2 microarrays. Values are the mean ± SEM.

Austin, TX) and labeled with biotinylated nucleotides using the BioArray kit (Enzo Diagnostics, Farmingdale, NY). For each sample, 2–3 mice were pooled, and for each time point, 3–6 samples were collected from separate experiments and prepared. The resulting labeled RNA was hybridized to U74Av2 microarrays (Affymetrix, Santa Clara, CA), which were read with an argon laser detector (GeneArray Scanner; Affymetrix). Raw hybridization intensities were processed into expression values using the robust multichip analysis method.

Sera were collected from 3 mice on days 0, 1, 3, 7, 12, and 18 during the above experiments. Synovial fluid samples were taken on day 7 by aspirating fluid from dissected ankles. Synovial fluid protein was obtained by centrifuging the samples for 5 minutes at 2,400g and collecting the supernatant. Sera

and synovial fluid protein were incubated in triplicate for 2 hours at room temperature in enzyme immunoassay/ radioimmunoassay plate wells (Corning, Corning, NY) that had been coated with 0.4 μg/ml of purified AF808 anti-osteopontin antibody (R&D Systems, Minneapolis, MN). Biotinylated BAF808 anti-osteopontin antibody (0.25 μg/ml; R&D Systems) was added for 1 hour at room temperature, then avidin–horseradish peroxidase secondary antibody (PharMingen, San Diego, CA) was added for an additional 1 hour at room temperature. Each of the antibody binding steps was followed by 5 washes with 0.05% Tween 20–PBS.

Substrate solution (TMB Substrate Reagent set; PharMingen) was added for 15–20 minutes at room temperature. The plates were then read on an ELISA plate reader (Molecular Devices, Sunnyvale, CA) at 450 nm. Recombinant mouse osteopontin (441-OP; R&D Systems) was used as a protein standard.
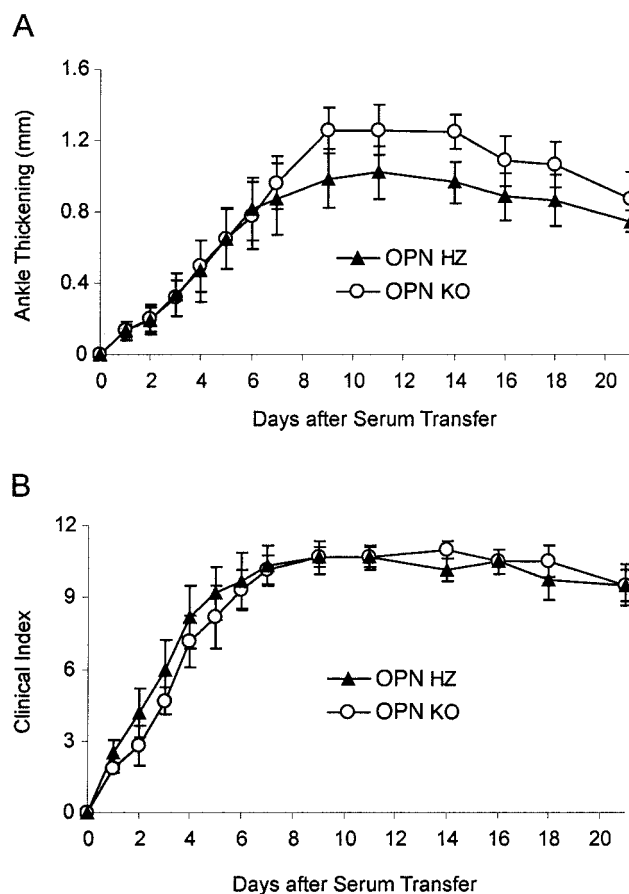

Figure 2. Comparable severity of arthritis in osteopontin (OPN)–deficient and OPN-sufficient mice upon injection with K/BxN serum. OPN-sufficient (heterozygous [HZ]) (n = 6) and OPN-deficient (KO) mice (n = 6) were injected with 150 μl of K/BxN serum on days 0 and 2 in 2 separate experiments. The ankle thickening **(A)** and clinical index **(B)** were measured from day 0 to day 15 (n = 2) or day 21 (n = 4). Values are the mean ± SEM. $P > 0.05$ at every time point.
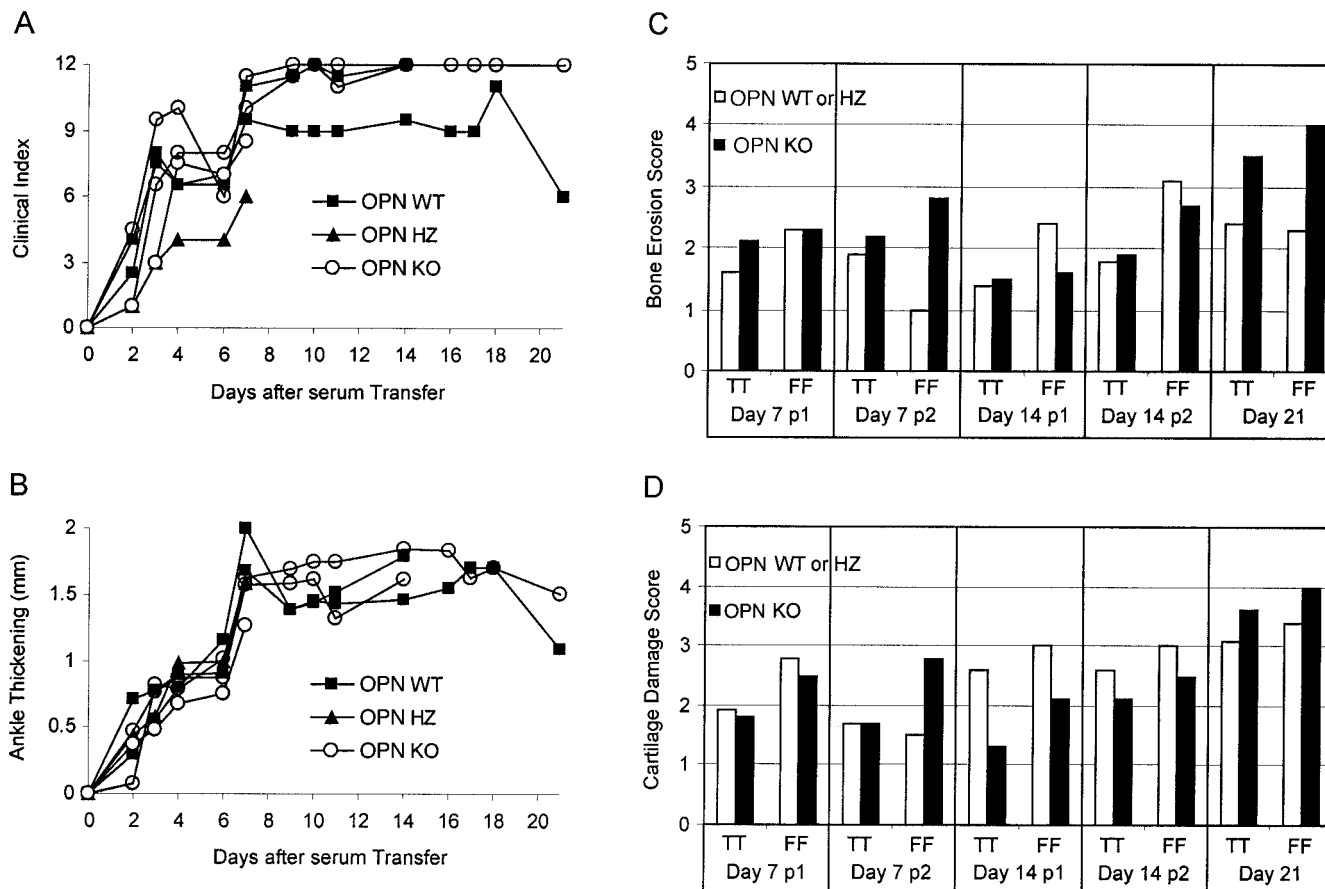
Figure 3. Comparable bone destruction and cartilage damage in the tibiotalar and forefoot regions of osteopontin (OPN)–sufficient and OPN-deficient mice. OPN-sufficient (wild-type [WT] or heterozygous [HZ]) and OPN-deficient (KO) mice (n = 3 per group) were injected with 150 μl of K/BxN serum on days 0, 2, 7, and 14. One mouse from each group was killed on days 7, 14, and 21 after initial K/BxN serum injection. The clinical index **(A)** and ankle thickening **(B)** were measured throughout the 3-week observation period. Histopathologic scoring of bone erosion **(C)** and cartilage damage **(D)** was performed on hind limbs from OPN-sufficient and OPN-deficient mice killed on days 7, 14, and 21. The limbs were compared in pairs (p) matched for left/right position. The tibiotalar (TT) and forefoot (FF) regions were scored separately.

Histologic assessment and histopathologic scoring. Hind limbs were prepared for histology by dissecting off the skin and outer muscle and then separating the knee and ankle joints at the mid-tibia. Specimens were fixed in 4% paraformaldehyde for a minimum of 12 hours and demineralized for ~2 weeks in 14% EDTA. Specimens were subsequently embedded in paraffin (Citadel 1000; Shandon, Pittsburgh, PA). For each specimen, at least 15 serial 5-μm sagittal sections were cut, and every fifth section was stained with hematoxylin and eosin (H&E; Sigma, St. Louis, MO) for evaluation of inflammation, bone erosion, and cartilage destruction. An adjacent section was stained with toluidine blue (Sigma) to assess cartilage integrity.

Staining for tartrate-resistant acid phosphatase (TRAP) was performed by a modification of a previously described method (30). Briefly, sections were incubated for 15 minutes at 37°C in freshly prepared 0.1 moles/liter Tris buffer, pH 5.0, 1.35 mmoles/liter naphthol-AS-MX phosphate

(Sigma), 0.362 moles/liter *N,N*-dimethylformamide, 3.88 mmoles/liter violet LB salt (Sigma), and 25 mmoles/liter sodium tartrate. Slides were rinsed for 10 minutes and counterstained with hematoxylin.

A total of 4–8 H&E-stained sections were scored by a blinded observer (ARP) at low power for inflammation and at low and high power for bone erosion. Inflammation was assessed in synovium and periarticular soft tissues of the ankle and forefoot. Each section was scored on a scale of 0–5 for inflammation (0 = normal, 1 = mild edema and/or minimal cellular infiltrate, 2 = mild cellular infiltrate, 3 = moderate cellular infiltrate, 4 = marked cellular infiltrate, and 5 = severe cellular infiltrate). Edema was included only in the minimal inflammation score since it is an early feature of this arthritis model (26). Bone erosion was assessed separately in the tibiotalar and forefoot regions and was scored on a 0–5 scale as previously described (31), with the minor modifications described previously (32). Cartilage was evaluated for
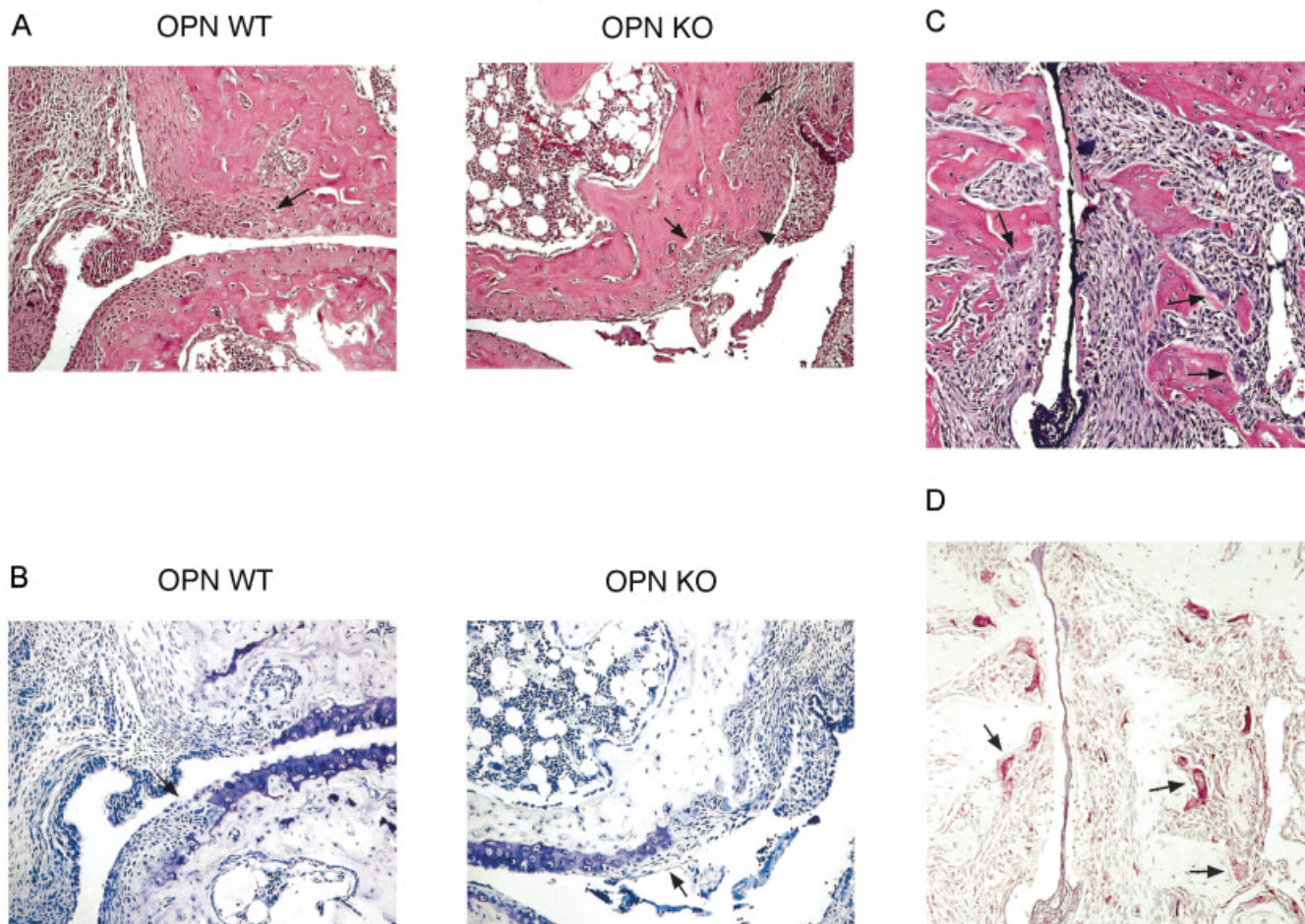
Figure 4. Comparable pannus formation, cartilage damage, and bone erosion in the arthritic joints of osteopontin (OPN)–deficient and wild-type mice. **A** and **B,** Representative sections of the tibiotalar region of matched OPN–wild-type (WT) and OPN-knockout (KO) mice 21 days after the initial injection of K/BxN serum. Inflammation, bone erosion (**arrows**), and formation of new woven bone (**arrowhead**) were evident on hematoxylin and eosin (H&E)–stained sections (**A**) of both the WT and KO mice. Pannus formation and associated cartilage destruction (**arrow**) were observed in toluidine blue–stained sections (**B**) of both the WT and KO mice. Cartilage damage and proteoglycan loss also occurred in areas remote from pannus. **C,** KO mice demonstrated bone erosion in H&E-stained sections of the forefoot region. **D,** Staining for tartrate-resistant acid phosphatase (TRAP) activity in a section adjacent to that in **C** revealed numerous TRAP+ multinucleated osteoclast-like cells at sites of bone erosion (**arrows** in **C** and **D**). (Original magnification × 50.)

surface damage and cartilage destruction secondary to pannus invasion as previously described (32). Cartilage damage was scored on serial toluidine blue–stained sections based upon proteoglycan loss in areas remote from inflamed synovium. Cartilage destruction in areas adjacent to pannus was scored separately on H&E-stained sections. The mean score for each histopathologic feature was calculated.

RESULTS

Local up-regulation of OPN mRNA during K/BxN serum-transferred arthritis. Given the interest in the role of OPN in inflammatory arthritis, we used microarray analysis of ankle synovial tissue taken at 6 time points to assess OPN mRNA expression during the unfolding of K/BxN serum-transferred arthritis (Jacobs JP: unpublished observations). OPN mRNA was progressively up-regulated in ankle joints as arthritis developed (Figure 1). Levels of OPN mRNA began to rise at about the time of clinical disease onset (day 3), reached an intermediate value as disease began to peak (day 7), and rose more slowly thereafter as the disease settled in (days 12 and 18). The concentration of OPN protein in synovial fluid on day 7 was 1.01 ± 0.26 μg/ml (mean $\pm$ SEM) as determined by ELISA. This level was 3.6-fold greater than the OPN concentration in serum taken

from the same mice (0.28 ± 0.02 μg/ml). The elevated OPN protein in synovial fluid suggests that OPN was produced locally in the joint, consistent with the mRNA increase. In contrast to the local up-regulation of OPN in ankles, no change in peripheral blood OPN mRNA expression or serum OPN protein was observed during K/BxN serum-transferred arthritis (data not shown).

Lack of protection of OPN deficiency against inflammation in serum-transferred arthritis at a range of disease severities. To test the physiologic relevance of OPN to K/BxN serum-transferred arthritis, we injected K/BxN serum into tenth-generation B6-backcrossed OPN-deficient mice and their matched OPN-sufficient littermates. The kinetics and severity of arthritis, as measured by ankle thickening and clinical index, in the wild-type and heterozygous OPN-sufficient mice were comparable with those in the OPN-knockout mice (Figures 2A and B and 3A and B).

Synovial inflammation was assessed in H&E-stained sections of the tibiotalar and forefoot regions. Thickening of the synovial lining, mononuclear cell infiltration, and pannus formation were observed both in OPN-sufficient and in OPN-deficient mice (Figure 4A). H&E-stained sections (n = 4) taken on day 15 were scored by a blinded observer on a 0–5 scale, corresponding to the degree of inflammatory cell infiltration into the ankles. Consistent with the clinical findings, OPN-sufficient and OPN-deficient mice had comparable histologic inflammation scores (2.8 ± 0.4 [mean ± SEM] versus 3.1 ± 0.2, respectively).

It remained possible that differences between OPN-sufficient and OPN-deficient mice were obscured because the system was overloaded. A dose-response experiment was performed using doses of K/BxN serum corresponding to a range of disease severities encompassing mild to severe arthritis. No differences in the clinical parameters of arthritis were observed between OPN-sufficient and OPN-deficient mice at any of the doses tested (Figure 5).

Comparable histologic features of bone erosion and cartilage damage in arthritic OPN-deficient and OPN-sufficient mice. We considered the possibility that OPN-deficient mice have the same level of inflammation as OPN-sufficient mice, but with reduced bone erosion and/or cartilage damage. H&E-stained sections of the tibiotalar region revealed bone erosions in both OPN-sufficient and OPN-deficient arthritic mice (Figure 4A). Bone erosion was also observed in the forefoot region of OPN-deficient mice (Figure 4C) and OPN-sufficient mice (results not shown), in some cases with marked trabecular and subchondral bone loss. Staining of serial

sections of the forefoot region for TRAP activity revealed numerous TRAP+ multinucleated osteoclast-like cells at sites of bone erosion in both groups (Figure 4D and data not shown). As has been reported previously with this model (33), we observed not only bone erosion, but also the formation of new woven bone undergoing remodeling by multinucleated osteoclast-like cells. This process was seen in the joints of both OPN-deficient (Figure 4A) and OPN-sufficient (results not shown) mice.

Damage to the cartilage was assessed on toluidine blue– and H&E-stained sections of the tibiotalar and forefoot regions. Invasion of the pannus into cartilage was associated with loss of cartilage matrix and proteoglycan content in OPN-sufficient and OPN-deficient mice (Figure 4B). Proteoglycan and cartilage matrix loss in areas distal from pannus also occurred in both groups (Figure 4B), as did full-depth cartilage loss, generally in association with loss of the underlying subchondral bone (Figure 4C and data not shown).

We directly compared the extent of bone erosion and cartilage damage in OPN-sufficient and OPN-deficient mice by semiquantitative histopathologic scoring. Sections were taken from mice killed 7, 14, or 21 days after the initial K/BxN serum injection. The forefoot and tibiotalar regions were evaluated independently to reflect the variation in disease severity that can occur in these areas. Bone erosion on H&E-stained sections was scored on a 0–5 scale. OPN-deficient and OPN-sufficient mice demonstrated comparable erosion in the tibiotalar and forefoot regions at the 3 time points (Figure 3C). The degree of bone erosion was generally proportionate to the degree of inflammation in all mice (data not shown), consistent with previously published observations (33). Surface cartilage damage and cartilage destruction secondary to pannus invasion were scored on toluidine blue– and H&E-stained sections, respectively. The degree of cartilage damage (Figure 3D) and pannus destruction of cartilage (data not shown) was similar in arthritic OPN-deficient and OPN-sufficient mice. Comparable histologic scores for bone erosion and cartilage damage were also found for arthritic OPN-sufficient and OPN-deficient mice killed on day 15 (data not shown).

DISCUSSION

OPN-deficient mice injected with K/BxN serum developed inflammation, cartilage damage, and bone erosion comparable with that of OPN-sufficient controls. Previous studies have examined the role of OPN in
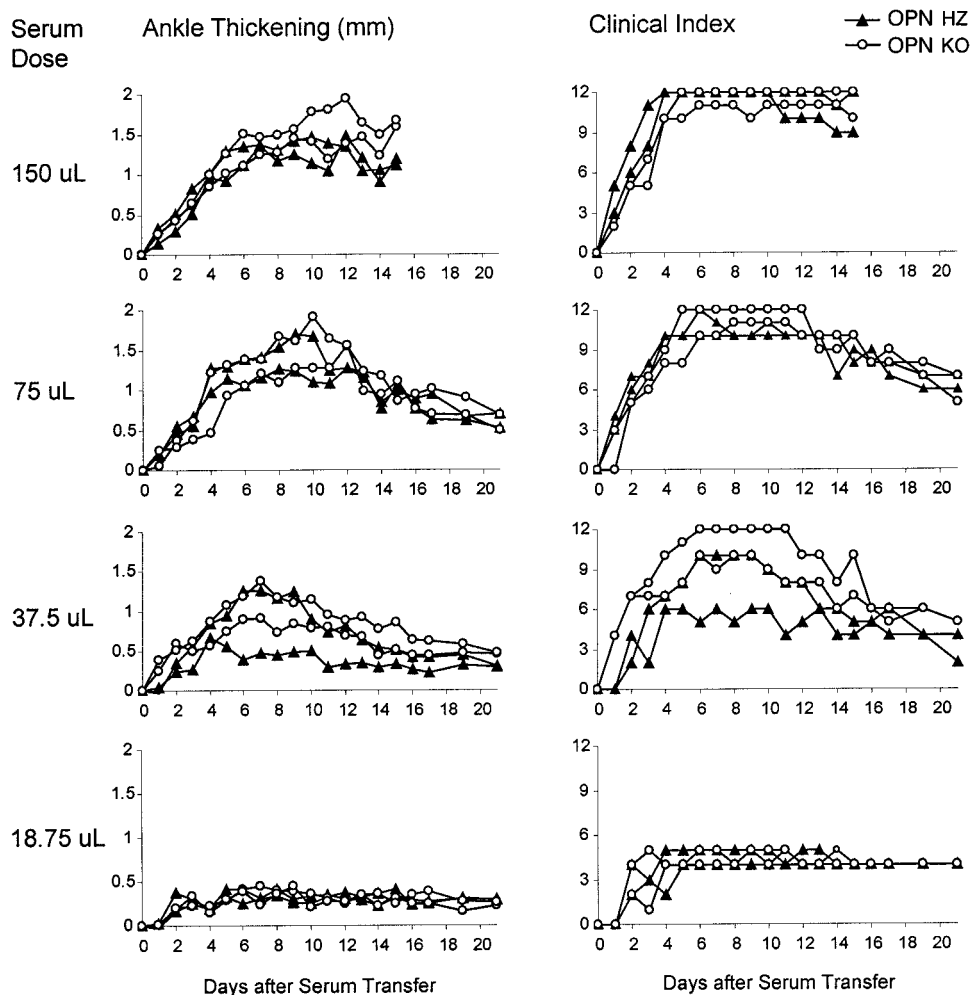
Figure 5. Similar response of osteopontin (OPN)–deficient and OPN-sufficient mice to a range of doses of K/BxN serum. OPN-sufficient (heterozygous [HZ]) and OPN-deficient (KO) mice were injected on days 0 and 2 with 150, 75, 37.5, or 18.75 μl of K/BxN serum (n = 2 mice at each dose). The ankle thickening and clinical index were measured from day 0 to day 15 (150-μl dose group) or day 21 (all other dose groups). Data for the 150-μl dose group were incorporated into Figure 2.

anti-CII antibody–induced arthritis (12,24). This model bears close mechanistic resemblance to K/BxN serum-transferred arthritis. In both systems, transferred auto-antibodies deposit in the joints and initiate pathogenic inflammation via complement activation and cellular innate immunity. Transfer experiments with various knockout mice have yielded largely concordant results between the two models, including demonstration of crucial roles for interleukin-1 receptor, C5a receptor, Fcγ receptor (FcγR), FcγRIIB, and FcγRIII (33–38). The similar pathogenic mechanisms suggest that the role of OPN in each model should also be comparable.

Our findings with the K/BxN serum-transfer model differed from those of Yumoto et al (24), who observed that OPN-deficient mice were resistant to anti-CII antibody–induced arthritis, but were consistent with those of Blom et al (12), who found no such protective effect. Both groups used similar experimental methods—cotransfer of anti-CII antibodies with lipo-polysaccharide (LPS) into OPN-sufficient and OPN-deficient mice—except that different sources of antibodies and different lines of OPN-knockout mice were used. It could have been argued that the system used by Blom et al was not a robust one because only 1 of 4 injected

OPN-heterozygous mice and 3 of 10 injected OPN-knockout mice developed arthritis. This was not true of our system, since we observed a 100% incidence of arthritis after K/BxN serum transfer and similar disease kinetics and severity within batches of serum. The difference between our results and those of Yumoto et al also cannot be attributed to overloading the system, since OPN-deficient and OPN-sufficient mice had comparable responses to a wide range of serum doses.

As also argued by Blom et al (12), the discordant results reported by Yumoto et al (24) might be attributable to polymorphic genes linked to OPN. Yumoto et al compared OPN-knockout mice and wild-type littermates of mixed B6/129Sv F_2 background. Such a comparison does not control for the potential confounding effects of 129Sv genes linked to the OPN mutant allele. OPN is situated in a region on mouse chromosome 5 that carries many genes that could influence an inflammatory disease, including a cluster of CXCL chemokines, and has been found to encode quantitative trait loci associated with arthritis and EAE (12). Blom et al used OPN-knockout mice backcrossed onto the C57BL/10 background for 12 generations in order to minimize the possibility of any linked genes having an effect. The OPN knockout mice used in our study were derived from the same mutant line used by Yumoto et al, but the mutation has been backcrossed onto the B6 background for 10 generations, thereby minimizing confounding influences of 129Sv genome segments.

Recently, Yamamoto et al (25) reported that prophylactic administration of polyclonal antibodies against the SLAYGLR epitope on thrombin-cleaved OPN moderately reduced the clinical severity of anti-CII antibody–induced arthritis and delayed its onset. The polyclonal antibodies also slowed the progression of disease when given therapeutically. These findings raise the possibility that when OPN is absent from development, it may not be required for inflammation, since other factors could compensate in its absence, but in settings where OPN is present, blocking OPN may have a modulatory effect on inflammation. Alternatively, the apparent role of OPN in these experiments may reflect the use of LPS to boost arthritis induction (39,40). LPS induces macrophage expression of OPN in vitro and could induce local expression of OPN within joints by synovial macrophages in anti-CII antibody–induced arthritis (41). Since LPS also activates coagulation, much of this OPN may be cleaved by thrombin (42). This conversion could facilitate the proinflammatory activity of OPN, since thrombin-cleaved, but not full-length, OPN induces monocyte migration in vitro (25). Since the

dose of anti-CII antibodies used in previous studies was insufficient to induce arthritis without LPS, inhibiting the effects of LPS would, by itself, suppress arthritis, even if the arthritogenic effects of the anti-CII antibodies are unaffected. Hence, our results and those of Yamamoto et al are consistent with OPN having an important mechanistic role downstream of LPS but not of autoantibodies.

OPN is potentially relevant to arthritis not only for its effects on inflammation, but also for its role in bone resorption. Bone resorption induced in vivo by experimental stresses, including estrogen withdrawal, ectopic transplantation, and mechanical stress, is attenuated in OPN-deficient bone (15,17,18). In culture, OPN-deficient bone showed reduced bone resorption and osteoclast differentiation in response to RANKL, an osteoclast differentiation factor that plays a central role in the regulation of bone remodeling (18,43). In K/BxN serum-transferred arthritis, RANKL-deficient mice had inflammation comparable with that of control mice, but had dramatically reduced bone erosion (32). If OPN is a downstream factor in osteoclast-mediated bone resorption, OPN-deficient mice injected with K/BxN serum would be expected to have diminished bone resorption compared with OPN-sufficient mice, even though they had comparable inflammation. In a routine histopathologic analysis of H&E-stained joint sections from OPN-deficient and OPN-sufficient arthritic mice, both groups demonstrated similar inflammation and bone resorption. Subtle differences in bone erosion between the two groups would not be detected in our analysis but might be observed using more sensitive methods of quantitation. Nevertheless, significant osteoclast-mediated bone erosion does occur in the absence of OPN in this arthritis model. We conclude that if OPN does act downstream of RANKL, it is not a necessary mediator of bone erosion in antibody-mediated arthritis.

We have shown that OPN does not play a required role in inflammation, bone erosion, or cartilage damage in the K/BxN serum-transfer model of autoantibody-mediated arthritis. Indeed, in the absence of OPN, these 3 parameters of arthritis are unaltered. It remains possible that OPN is involved in the recruitment of inflammatory cells or in bone erosion during arthritis but the effects of its absence are too small to be detected in our analysis, or that in its absence, other factors compensate. In either scenario, OPN by itself is not an attractive target for reducing inflammation and bone destruction in RA patients.

ACKNOWLEDGMENTS

REFERENCES

1. Denhardt DT, Noda M. Osteopontin expression and function: role in bone remodeling. J Cell Biochem Suppl 1998;30:92–102.
2. O'Regan A, Berman JS. Osteopontin: a key cytokine in cell-mediated and granulomatous inflammation. Int J Exp Pathol 2000;81:373–90.
3. Denhardt DT, Noda M, O'Regan AW, Pavlin D, Berman JS. Osteopontin as a means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival. J Clin Invest 2001;107:1055–61.
4. Giachelli CM, Lombardi D, Johnson RJ, Murray CE, Almeida M. Evidence for a role of osteopontin in macrophage infiltration in response to pathological stimuli in vivo. Am J Pathol 1998;152:353–8.
5. Weiss JM, Renkl AC, Maier CS, Immig M, Liaw L, Ahrens T, et al. Osteopontin is involved in the initiation of cutaneous contact hypersensitivity by inducing Langerhans and dendritic cell migration to lymph nodes. J Exp Med 2001;194:1219–29.
6. Ashkar S, Weber GF, Panoutsakopoulou V, Sanchirico ME, Jansson M, Zawaideh S, et al. Eta-1 (osteopontin): an early component of type-1 (cell-mediated) immunity. Science 2000;287:860–4.
7. O'Regan AW, Chupp GL, Lowry JA, Goetschkes M, Mulligan N, Berman JS. Osteopontin is associated with T cells in sarcoid granulomas and has T cell adhesive and cytokine-like properties in vitro. J Immunol 1999;162:1024–31.
8. Lampe MA, Patarca R, Iregui MV, Cantor H. Polyclonal B cell activation by the Eta-1 cytokine and the development of systemic autoimmune disease. J Immunol 1991;147:2902–6.
9. Iizuka J, Katagiri Y, Tada N, Murakami M, Ikeda T, Sato M, et al. Introduction of an osteopontin gene confers the increase in B1 cell population and the production of anti-DNA autoantibodies. Lab Invest 1998;78:1523–33.
10. Chabas D, Baranzini SE, Mitchell D, Bernard CC, Rittling SR, Denhardt DT, et al. The influence of the proinflammatory cytokine, osteopontin, on autoimmune demyelinating disease. Science 2001;294:1731–5.
11. Jansson M, Panoutsakopoulou V, Baker J, Klein L, Cantor H. Cutting edge: attenuated experimental autoimmune encephalomyelitis in Eta-1/osteopontin-deficient mice. J Immunol 2002;168:2096–9.
12. Blom T, Franzen A, Heinegard D, Holmdahl R. Comment on the influence of the proinflammatory cytokine, osteopontin, on autoimmune demyelinating disease [letter]. Science 2003;299:1845.
13. Rittling SR, Matsumoto HN, McKee MD, Nanci A, An X, Novick KE, et al. Mice lacking osteopontin show normal development and bone structure but display altered osteoclast formation in vitro. J Bone Miner Res 1998;13:1101–11.
14. Liaw L, Birk DE, Ballas CB, Whitsitt JS, Davidson JM, Hogan BL. Altered wound healing in mice lacking a functional osteopontin gene (spp1). J Clin Invest 1998;101:1468–78.
15. Yoshitake H, Rittling SR, Denhardt DT, Noda M. Osteopontin-deficient mice are resistant to ovariectomy-induced bone resorption. Proc Natl Acad Sci U S A 1999;96:8156–60.
16. Chellaiah MA, Kizer N, Biswas R, Alvarez U, Strauss-Schoenberger J, Rifas L, et al. Osteopontin deficiency produces osteoclast dysfunction due to reduced CD44 surface expression. Mol Biol Cell 2003;14:173–89.
17. Ishijima M, Rittling SR, Denhardt DT, Noda M. Enhancement of osteoclastic bone resorption and suppression of osteoblastic bone formation in response to reduced mechanical stress do not occur in the absence of osteopontin. Proc Natl Acad Sci U S A 1999;96:8156–60.
18. Asou Y, Rittling SR, Yoshitake H, Tsuji K, Shinomiya K, Nifuji A, et al. Osteopontin facilitates angiogenesis, accumulation of osteoclasts, and resorption in ectopic bone. Endocrinology 2001;142:1325–32.
19. Ihara H, Denhardt DT, Furuya K, Yamashita T, Muguruma Y, Tsuji K, et al. Parathyroid hormone-induced bone resorption does not occur in the absence of osteopontin. J Biol Chem 2001;276:13065–71.
20. Gravallese EM. Osteopontin: a bridge between bone and the immune system. J Clin Invest 2003;112:147–9.
21. Petrow PK, Hummel KM, Schedel J, Franz JK, Klein CL, Muller-Ladner U, et al. Expression of osteopontin messenger RNA and protein in rheumatoid arthritis. Arthritis Rheum 2000;43:1597–605.
22. Ohshima S, Yamaguchi N, Nishioka K, Mima T, Ishii T, Umeshita-Sasai M, et al. Enhanced local production of osteopontin messenger RNA and protein in rheumatoid arthritis. J Rheumatol 2002;29:2061–7.
23. Ohshima S, Kobayashi H, Yamaguchi N, Nishioka K, Umeshita-Sasai M, Mima T, et al. Expression of osteopontin at sites of bone erosion in a murine experimental arthritis model of collagen-induced arthritis. Arthritis Rheum 2002;46:1094–101.
24. Yumoto K, Ishijima M, Rittling SR, Tsuji K, Tsuchiya Y, Kon S, et al. Osteopontin deficiency protects joints against destruction in anti-type II collagen antibody-induced arthritis in mice. Proc Natl Acad Sci U S A 2002;99:4556–61.
25. Yamamoto N, Sakai F, Kon S, Morimoto J, Kimura C, Yamazaki H, et al. Essential role of the cryptic epitope SLAYGLR within osteopontin in a murine model of rheumatoid arthritis. J Clin Invest 2003;112:181–8.
26. Kouskoff V, Korganow AS, Duchatelle V, Degott C, Benoist C, Mathis D. Organ-specific disease provoked by systemic autoreactivity. Cell 1996;87:811–22.
27. Korganow AS, Ji H, Mangialaio S, Duchatelle V, Pelanda R, Martin T, et al. From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. Immunity 1999;10:451–61.
28. Matsumoto I, Staub A, Benoist C, Mathis D. Arthritis provoked by linked T and B cell recognition of a glycolytic enzyme. Science 1999;286:1732–5.
29. Matsumoto I, Maccioni M, Lee DM, Maurice M, Simmons B, Brenner M, et al. How antibodies to a ubiquitous cytoplasmic enzyme may provoke joint-specific autoimmune disease. Nat Immunol 2002;3:360–5.
30. Glowacki J, Rey C, Glimcher MJ, Cox KA, Lian J. A role for osteocalcin in osteoclast differentiation. J Cell Biochem 1991;45:292–302.
31. Bendele A, McAbee T, Sennello G, Frazier J, Chlipala E, McCabe D. Efficacy of sustained blood levels of interleukin-1 receptor antagonist in animal models of arthritis: comparison of efficacy in animal models with human clinical data. Arthritis Rheum 1999;42:498–506.
32. Pettit AR, Ji H, von Stechow D, Muller R, Goldring SR, Choi Y, et al. TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. Am J Pathol 2001;159:1689–99.
33. Ji H, Pettit A, Ohmura K, Ortiz-Lopez A, Duchatelle V, Degott C, et al. Critical roles for interleukin-1 and tumor necrosis factor-α in antibody-induced arthritis. J Exp Med 2002;196:77–85.
34. Ji H, Gauguier D, Ohmura K, Gonzalez A, Duchatelle V, Danoy

P, et al. Genetic influences on the end-stage effector phase of arthritis. J Exp Med 2001;194:321–30.

35. Ji H, Ohmura K, Mahmood U, Lee DM, Hofhuis FM, Boackle SA, et al. Arthritis critically dependent on innate immune system players. Immunity 2002;16:157–68.

36. Kagari T, Doi H, Shimozato T. The importance of IL-1β and TNF-α, and the noninvolvement of IL-6, in the development of monoclonal antibody-induced arthritis. J Immunol 2002;169:1459–66.

37. Grant EP, Picarella D, Burwell T, Delaney T, Croci A, Avitahl N, et al. Essential role for the C5a receptor in regulating the effector phase of synovial infiltration and joint destruction in experimental arthritis. J Exp Med 2002;196:1461–71.

38. Kagari T, Tanaka D, Doi H, Shimozato T. Essential role of Fcγ receptors in anti-type II collagen antibody-induced arthritis. J Immunol 2003;170:4318–24.

39. Terato K, Hasty KA, Reife RA, Cremer MA, Kang AH, Stuart JM. Induction of arthritis with monoclonal antibodies to collagen. J Immunol 1992;148:2103–8.

40. Terato K, Harper DS, Griffiths MM, Hasty DL, Ye XJ, Cremer MA, et al. Collagen-induced arthritis in mice: synergistic effect of E. coli lipopolysaccharide bypasses epitope specificity in the induction of arthritis with monoclonal antibodies to type II collagen. Autoimmunity 1995;22:137–47.

41. Guo H, Cai CQ, Schroeder RA, Kuo PC. Osteopontin is a negative feedback regulator of nitric oxide synthesis in murine macrophages. J Immunol 2001;166:1079–86.

42. Okajima K. Regulation of inflammatory responses by natural anticoagulants. Immunol Rev 2001;184:258–74.

43. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature 2003;423:337–42.