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Particularities of the vasculature can promote the organ specificity of autoimmune attack

Bryce A Binstadt[1,2,3], Pratik R Patel[4,6], Herlen Alencar[1,4], Peter A Nigrovic[1,3,5], David M Lee[1,5], Umar Mahmood[1,4], Ralph Weissleder[1,4], Diane Mathis[1,2,5] & Christophe Benoist[1,2,5]

How certain autoimmune diseases target specific organs remains obscure. In the 'K/BxN' arthritis model, autoantibodies to a ubiquitous antigen elicit joint-restricted pathology. Here we have used intravital imaging to demonstrate that transfer of arthritogenic antibodies caused macromolecular vasopermeability localized to sites destined to develop arthritis, augmenting its severity. Vasopermeability depended on mast cells, neutrophils and FcγRIII but not complement, tumor necrosis factor or interleukin 1. Unexpectedly, radioresistant FcRγ-expressing cells in an organ distant from the joint were required. Histamine and serotonin were critical, and systemic administration of these vasoactive amines recapitulated the joint localization of immune complex–triggered vasopermeability. We propose that regionally distinct vascular properties 'interface' with immune effector pathways to foster organ-specific autoimmune damage, perhaps explaining why arthritis accompanies many human infectious and autoimmune disorders.

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In most immunology textbooks, the long list of autoimmune diseases is represented as dichotomous, with individual disorders considered to be either systemic or organ-specific. The prevailing explanation for the restriction of pathological manifestations to a single organ is that T and/or B lymphocytes capable of recognizing an antigen synthesized by one of the body's parenchymal cell types somehow escape the normal network of central and peripheral tolerance mechanisms and attack the organ containing that kind of cell. To the extent that the antigen is uniquely expressed by that cell type and that those cells reside uniquely in that particular organ, the eventual outcome is an organ-specific autoimmune disease. Although there is no doubt that this paradigm is valid, evidence is emerging that restriction of pathology to one or a few organs can be achieved by additional means[1–3]. Unfortunately, there are few clues now about what these alternative mechanisms might be.

One example is provided by the 'K/BxN' T cell receptor–transgenic mouse model of inflammatory arthritis. The development of disease in this strain depends on the reactivity of T lymphocytes (bearing the transgene-encoded T cell receptor 'KRN') and B lymphocytes to a ubiquitously expressed self antigen, glucose-6-phosphate isomerase (GPI)[1,4]. Transfer of serum containing autoantibodies to GPI, mainly of the immunoglobulin G1 (IgG1) isotype, rapidly elicits arthritis in healthy recipient mice, in particular at the distal joints, even in the absence of T cells and B cells[5]. When administered alone, monoclonal antibodies to GPI do not cause arthritis, whereas various combinations of them do promote disease, provided that they recognize spaced epitopes[6], suggesting that immune complex formation is essential for the generation of arthritis. Many elements of the innate immune system have been reported to be key effectors in the K/BxN serum-transfer system, including the alternative pathway of complement, the low-affinity IgG receptor (FcγRIII), mast cells, neutrophils and the proinflammatory cytokines interleukin 1 and tumor necrosis factor[7–11].

An obvious issue is why the autoimmune inflammatory attack in K/BxN mice is focused on the distal joints. The solution seems to lie not in the disease-initiation phase, dependent on the adaptive immune system, because the autoreactive T cells and B cells, as well as the autoantibodies produced by the latter, recognize the ubiquitously expressed antigen GPI[4]. In addition, no special features of the concentration or form of joint-localized GPI have been detected[12], and naive GPI-reactive T cells proliferate equally well in all lymph nodes after transfer into healthy recipients of the appropriate major histocompatibility complex haplotype[13]. Indeed, the effector stage of the disease, as recapitulated in the K/BxN serum-transfer system, is itself joint focused and also 'preferentially' affects the distal extremities[5]. At first glance, the identities of the chief participants in this innate immune system response also fail to provide an answer. In fact, they form a group of elements (immune complexes, complement, FcγRIII, mast cells, neutrophils tumor necrosis factor, interleukin 1) very reminiscent of those critical during the type III hypersensitivity Arthus reaction, which can manifest at many sites in the body.

[1]Harvard Medical School, Boston, Massachusetts 02115, USA. [2]Section on Immunology and Immunogenetics, Joslin Diabetes Center, Boston, Massachusetts 02215, USA. [3]Rheumatology Program, Children's Hospital Boston, Boston, Massachusetts 02115, USA. [4]Center for Molecular Imaging Research, Massachusetts General Hospital, Charlestown, Massachusetts 02129, USA. [5]Division of Rheumatology, Immunology and Allergy and Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts 02115, USA. [6]Present address: Novartis Institutes for BioMedical Research, Cambridge, Massachusetts 02139, USA. Correspondence should be addressed to D.M. (cbdm@joslin.harvard.edu) or C.B. (cbdm@joslin.harvard.edu).

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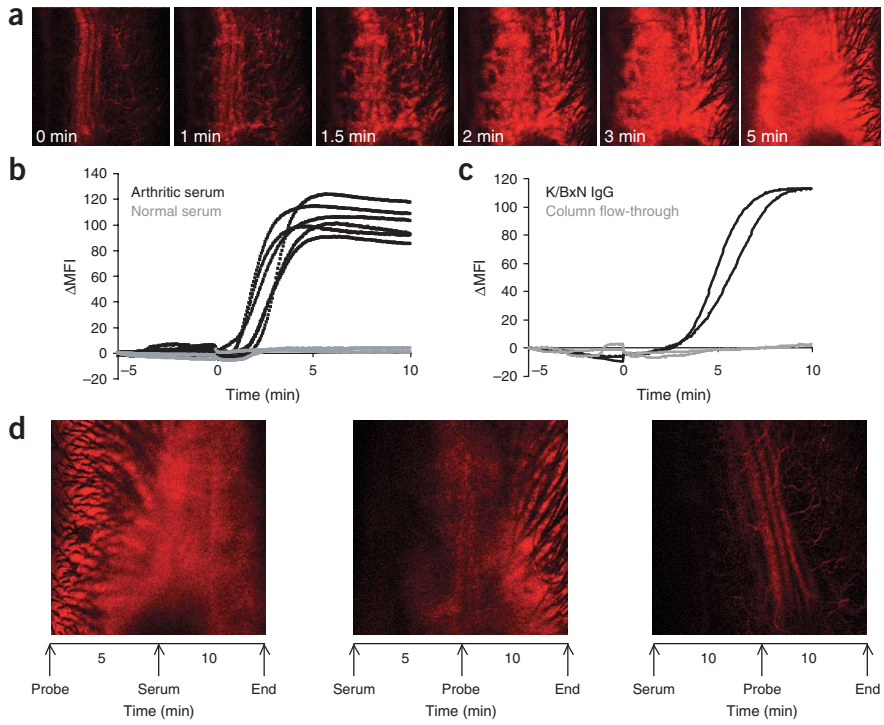

Figure 1 IgG from K/BxN arthritic serum induces rapid and brief vascular leak. The vascular probe AngioSense 680 was injected intravenously into B6 mice and was allowed to circulate for 5 min, followed by intravenous injection of various agents. (**a**) Single-plane confocal micrographs of a hindpaw at various times after injection of 200 μl of arthritogenic K/BxN serum (**Supplementary Video 1** online). (**b**) Quantification of the change in mean fluorescence intensity (ΔMFI) of confocal micrographs obtained every 3 s for 5 min before (–5) and 10 min after injection (at time 0) of 200 μl of either arthritogenic K/BxN serum (Arthritic serum) or nonarthritogenic (BxN) serum (Normal serum). ΔMFI was calculated relative to the MFI 5 min before injection for all experiments. Results (**a**,**b**) are representative of experiments with more than 20 mice injected with K/BxN serum and 3 mice injected with BxN serum. (**c**) Quantification of the ΔMFI of confocal micrographs obtained every 3 s for 5 min before (–5) and 10 min after injection (at time 0) of 200 μl of protein G–purified IgG from K/BxN serum (K/BxN IgG; $n = 2$) or the nonbound fraction (Column flow-through; $n = 2$). (**d**) Confocal micrographs of hindpaws after intravenous injection of 200 μl of K/BxN serum 5 min after (left), 5 min before (middle) or 10 min before (right) injection of the vascular probe. Images were obtained 10 min after the second injection. Original magnification, ×4.

Results from the experiments described here demonstrate an additional mechanism capable of promoting organ-specific autoimmune inflammatory attack: vascular leakage localized to the distal extremities in response to immune complexes circulating systemically in the blood. Delineation of the mechanisms underlying this restricted macromolecular vasopermeability has indicated involvement of unexpected participants. These include the vasoactive amines histamine and serotonin and an organ distant from the joint, most likely the liver or the gut.

RESULTS

Arthritogenic antibodies cause localized vascular leak

As changes in the microvasculature are one of the earliest detectable antecedents of inflammation[14], we sought to monitor blood vessel alterations in recipients of K/BxN serum. For this, we devised a noninvasive, real-time method for imaging the microvasculature of live mice using confocal microscopy and a new polyethylene glycol–conjugated intravascular imaging probe, AngioSense 680 (molecular mass, 400 kilodaltons)[15]. This fluorescent probe remains stable in the circulation of a normal mouse for several hours, rendering it useful for visualizing both the vascular bed and macromolecular leakage from the vessels. Intravenous administration of serum from an arthritic K/BxN mouse to normal C57BL/6 (B6) mice resulted in rapid extravasation of the vascular probe in the paws (**Fig. 1a** and **Supplementary Video 1** online), with reproducible kinetics and severity (**Fig. 1b**). Injection of normal mouse serum or IgG-depleted arthritogenic serum did not appreciably increase vasopermeability (**Fig. 1b**,**c**); instead, leakage depended on the serum IgG fraction (**Fig. 1c**).

The increase in vascular leakage occurred rapidly after injection of K/BxN serum. Analysis of the rate curves of 17 B6 mice (**Supplementary Fig. 1** online) showed that macromolecular extravasation reached its maximum rate 2.66 ± 0.18 min after serum administration. The time during which the rate of leakage was 50% or more of the maximum rate was 2.03 ± 0.08 min, suggesting that the total duration was approximately 4 min. We therefore reasoned that the entire observable phenomenon would cease about 7 min after serum injection. We tested that computational prediction experimentally and found that vascular probe injected 5 min after serum administration did extravasate, although detectably less than when given before serum, whereas probe injected 10 min after serum administration did not leak detectably (**Fig. 1d**). Thus, the vascular response demonstrated by our assay is rapidly induced and short-lived.

Although we administered the serum intravenously into the systemic circulation, the increase in macromolecular leak was anatomically restricted, mainly to the paws (**Fig. 2a**). Vessels in the skin of the back or on the surface of the cecum showed minimal extravasation of the vascular probe. Some mice had small foci of increased vasopermeability in the vessels of the external ear, but this effect was less reproducible and less vigorous than the response in the paws. These findings collectively demonstrate that one of the initial events of 'serum-transferred arthritis' is a rapid increase in macromolecular vasopermeability localized to the distal extremities.

Nonspecific immune complexes cause local vascular leak

We next sought to determine whether the vascular response described above was dependent on specific antibody recognition of the target antigen in the K/BxN model (GPI) or whether nonspecific antibodies might also produce such a response. As GPI-specific antibodies are known to form complexes with GPI circulating in the serum[12], we first evaluated the activity of nonspecific immune complexes. Intravenous administration of preaggregated normal mouse IgG elicited an increase in joint-localized vasopermeability very similar to that induced by the administration of arthitogenic serum (**Fig. 2b** and data not shown), which was also joint localized (data not shown). In contrast, monomeric mouse IgG did not induce macromolecular leakage (**Fig. 2b**). Furthermore, vasopermeability could be induced by

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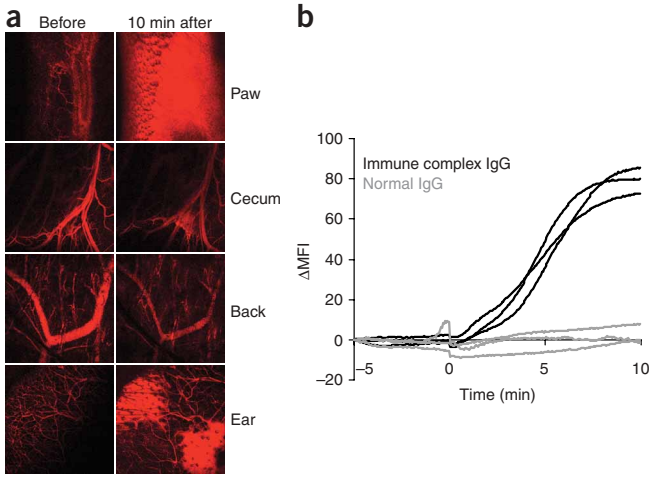

Figure 2 Vascular leak is tissue restricted but antigen nonspecific. (**a**) Confocal micrographs of the hindpaw (top row), cecum (second row), skin of the back (third row) and ear (bottom row) of a B6 mouse injected intravenously with AngioSense 680; this was allowed to circulate for 5 min, followed by intravenous injection of 200 μl of arthritogenic K/BxN serum. Images were obtained immediately before (left) and 10 min after (right) serum injection. Original magnification, ×4. Results are representative of at least four mice per site. (**b**) The calculated ΔMFI of confocal micrographs of the hindpaws of B6 mice injected intravenously with AngioSense 680; this was allowed to circulate for 5 min, followed by intravenous injection at time 0 of 100 μg of either normal mouse IgG (Normal IgG; $n = 3$) or heat-aggregated normal mouse IgG (Immune complex IgG; $n = 3$); images were obtained every 3 s.

immune complexes of mouse IgG1, IgG2a or IgG2b but not IgG3 (**Supplementary Fig. 2** online).

Cellular and molecular requirements

To begin determining the mechanisms underlying the increase in vasopermeability elicited by K/BxN serum, we assayed a panel of recipient mouse strains deficient in elements that have been identified as being required for arthritis induction. Mice genetically devoid of the complement component C3, the complement C5a receptor, the neonatal Fc receptor, the interleukin 1 receptor and tumor necrosis factor are fully or partially resistant to 'serum-transfer arthritis'[7,8,16]. However, the vascular response in these mice was indistinguishable from that of wild-type control mice (**Fig. 3a**). In contrast, in arthritis-resistant mice genetically lacking mast cells[10], neutrophils (P. Monach, D.M. and C.B., unpublished data) and the low-affinity IgG Fc receptor (FcγRIII) or its cytoplasmic signaling chain (FcRγ)[7], the immediate vascular response was greatly diminished (**Fig. 3b**). By histological evaluation, neutrophil-deficient mice had numbers of synovial mast cells equivalent to those of their wild-type littermates, and bone marrow mast cells cultured from neutrophil-deficient mice released β-hexoasaminidase normally in response to stimulation with IgE (data not shown). The high-affinity IgE receptor FcεRI also uses FcRγ for signal transduction, but mice lacking the Fc-binding chain FcεRIα had a normal vascular response (**Fig. 3a**) and also developed an arthritis similar to that of the control BALB/c strain (P.A.N., unpublished data). Thus, only a subset of the cellular and molecular elements required for the development of arthritis in recipients of K/BxN serum was needed to promote macromolecular leakage in the joint locale: signaling through the Fc receptor was essential, but complement activation, whose effect may be later with the potent chemotactic activity of the C5a product, was not. The vascular phenomenon involved the coordinated action of several cell types, as mast cells and neutrophils were both required.

We sought mechanistic insight from the identification of the FcRγ-bearing (or, more precisely, FcγRIII-bearing) cellular sensor of immune complexes. As a first step, we reconstituted irradiated B6

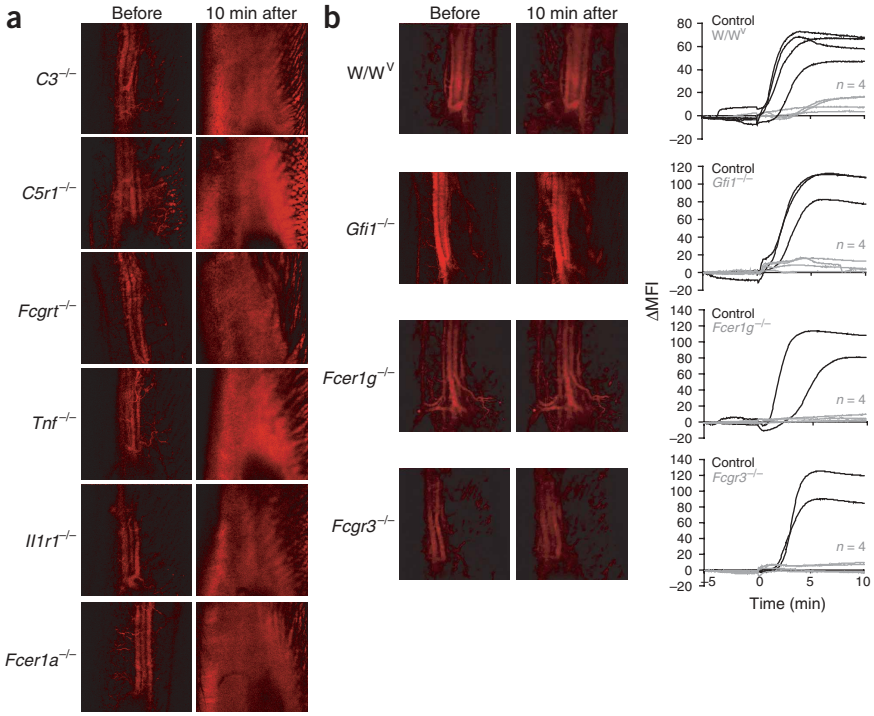

Figure 3 Molecular and cellular mediators of vascular leak. (**a**) Confocal micrographs of the hindpaws of mice injected intravenously with AngioSense 680. After 5 min, 200 μl of arthritogenic K/BxN serum was injected; images were obtained immediately before (left) and 10 min after (right) serum injection. Mice genetically lacked complement component C3 (*C3*$^{-/-}$), the receptor for complement component C5a (*C5r1*$^{-/-}$), the neonatal Fc receptor (*Fcgrt*$^{-/-}$), tumor necrosis factor (*Tnf*$^{-/-}$), interleukin 1 receptor (*Il1r1*$^{-/-}$) or FcεRIα (*Fcer1a*$^{-/-}$); $n = 3$–4 mice per group. (**b**) The calculated ΔMFI (right) of confocal micrographs (left and middle) of the hindpaws of mice injected intravenously with AngioSense 680. This was allowed to circulate for 5 min, followed by injection (at time 0) of 200 μl of arthritogenic K/BxN serum. Images of the hindpaws were obtained every 3 s before (left) and 10 min after (middle) serum injection. Mice were mast cell deficient (W/W^{v}), neutrophil deficient (*Gfi1*$^{-/-}$), devoid of FcRγ (*Fcer1g*$^{-/-}$) or devoid of FcγRIII (*Fcgr3*$^{-/-}$); control mice were WBB6 mice for W/W^{v} mice lacking mast cells; *Gfi1*$^{+/+}$ littermates for neutrophil-deficient (*Gfi1*$^{-/-}$) mice; and B6 mice for mice devoid of FcRγ or FcγRIII. Original magnification, ×4.

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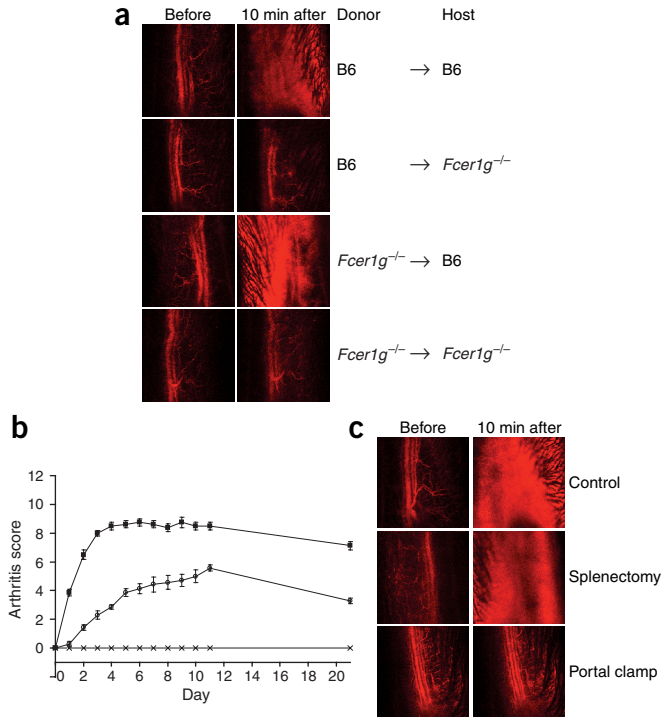

Figure 4 The site of FcRγ expression influences vascular response and arthritis severity. (**a**) Confocal micrographs of the hindpaws of radiation bone marrow chimera mice generated in a 'criss-cross' way (right): B6 donor and host; B6 donor, with host devoid of FcRγ (*Fcer1g*$^{-/-}$); donor devoid of FcRγ, with B6 host; or donor and host both devoid of FcRγ. Images were obtained before (left) and 10 min after (right) injection of 200 μl of arthritogenic K/BxN serum. Results are representative of six mice per group. (**b**) Clinical arthritis severity of radiation bone marrow chimera mice injected intravenously with 200 μl of K/BxN serum 12 weeks after transplant. Arthritis was assessed daily, as was ankle thickness (data not shown). ■, B6 donor and host ($n = 8$); ○, B6 donor with host devoid of FcRγ ($n = 7$); ×, donor devoid of FcRγ with B6 host ($n = 2$) or donor and host both devoid of FcRγ ($n = 2$; data are identical for these two groups). Data are means and s.e.m from one experiment. Results represent one of two independent experiments with a total of at least six mice per group. (**c**) Confocal micrographs of the hindpaws of B6 mice that underwent surgical splenectomy or ligation of the hepatic portal circulation (Portal clamp). Images were obtained before (left) and 10 min after (right) injection of 200 μl of K/BxN serum. Controls are unmanipulated mice. Results are representative of at least three mice per procedure. Original magnification, ×4.

or FcRγ-deficient mice with bone marrow from either B6 or FcRγ-deficient mice, in a 'criss-cross' way, and assessed the vascular leak response to arthritogenic serum 3 weeks later. Unexpectedly, regardless of the origin of the donor-derived compartment, host mice of B6 background demonstrated the vascular response, whereas hosts lacking FcRγ did not (**Fig. 4a**). We obtained identical results 12 weeks after bone marrow reconstitution (data not shown), suggesting that the FcRγ-bearing immune complex sensor resides in the radioresistant organs of the mouse rather than in the reconstituted hematopoietic compartment.

That finding was unexpected, given reports that arthritis development subsequent to K/BxN serum transfer depends on FcRγ expression on radiosensitive cells rather than on stromal cells[11]. We confirmed that although the vascular leak required FcRγ expression on radioresistant host cells, the development of arthritis depended more on the display of FcRγ on donor cells (**Fig. 4b**). However, mice susceptible to enhanced macromolecular extravasation (B6 donor and host mice) had a 'brisker tempo' of arthritis induction and greater arthritis severity than did mice with no augmented leak (B6 donor and FcRγ-deficient host mice), which is consistent with closer examination of the earlier report[11]. Histological examination of the latter combination confirmed a less severe disease process (data not shown). These findings suggest that the vascular leak response may not be completely essential for arthritogenesis but it serves an important amplifying function in early stages. Such an amplifying function is likely to be most important in suboptimal conditions, such as those probably encountered during the prolonged initiation of spontaneous diseases such as human rheumatoid arthritis, for which such a difference might determine whether or not clinical disease develops.

The first stromal cell candidates to be considered as potential immune complex sensors are endothelial cells in the vicinity of the joint. However, an important function for the liver and spleen in immune complex clearance has been recognized for decades[17]. We therefore attempted to determine whether circulation through those organs is necessary for the vascular leak promoted by serum from K/BxN mice. Splenectomized mice retained the vascular response to injection of arthritogenic serum, whereas there was no increase in

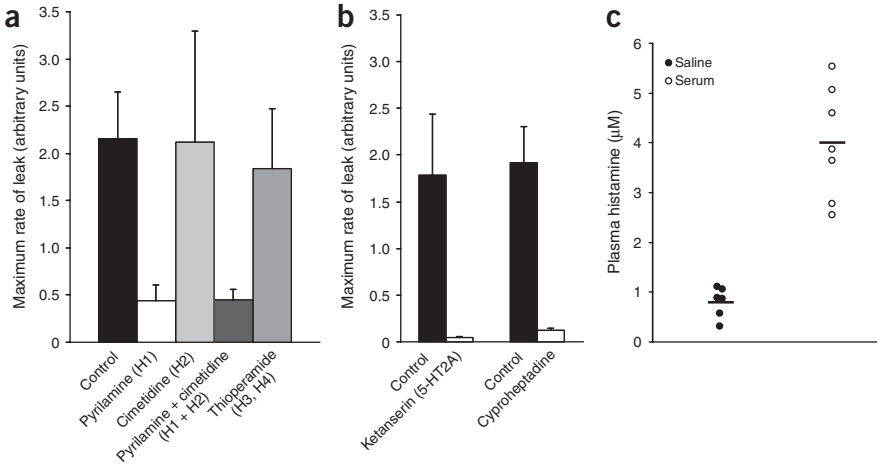

Figure 5 Vascular leak is blocked by histamine and serotonin receptor blockade. (**a**,**b**) Vascular leak in B6 mice treated with histamine receptor antagonists (**a**) or ketanserin or cyproheptadine (**b**), followed by induction of vascular leak with 200 μl of K/BxN serum. The maximum rate of leak (arbitrary units) was determined by using the first derivative of rate curves generated by plotting of the mean fluorescence intensity of individual confocal micrographs obtained every 3 s after serum injection (**Supplementary Fig. 1** online). Results are plotted as mean and s.e.m.; $n = 3$–4 mice per group. Control, normal saline. (**c**) Plasma histamine concentrations 5 min after treatment of B6 mice with normal saline (left) or arthritogenic K/BxN serum (right). Each circle represents an individual mouse and results are compiled from two independent experiments with three to four mice per group. Small horizontal bars indicate mean values.

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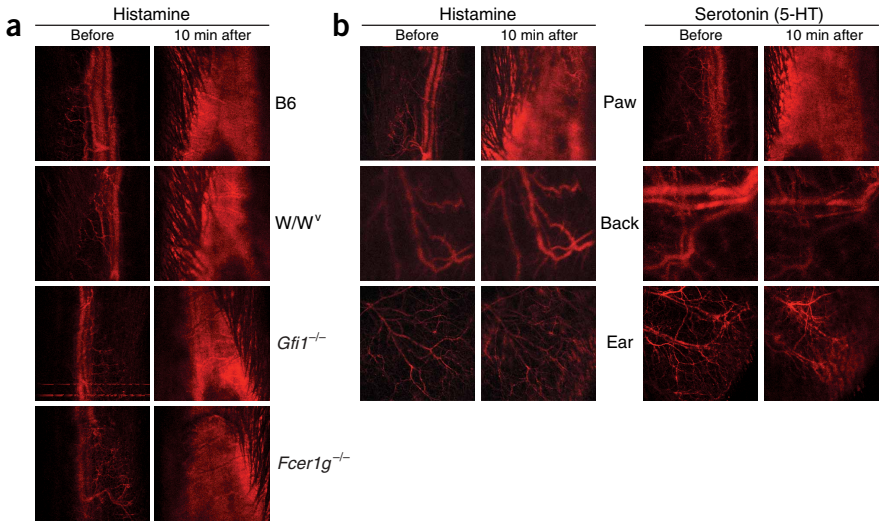

Figure 6 Vasoactive amines directly cause anatomically restricted vascular leak. (**a**) Representative confocal micrographs of hindpaws, obtained before (left) and 10 min after (right) intravenous injection of 20 mg/kg of histamine dihydrochloride into B6 mice (B6), mice lacking mast cells (W/W^v), neutrophil-deficient mice (*Gfi1*$^{-/-}$) and mice devoid of FcRγ (*Fcer1g*$^{-/-}$); $n = 3$ mice/group. (**b**) Representative confocal micrographs of the hindpaw (top row), back (middle row) and ear (bottom row) before (left in each) and 10 min after (right in each) intravenous injection of 20 mg/kg of histamine dihydrochloride (left two columns) or 10 mg/kg of serotonin (right two columns) into B6 mice. Images are representative of three to four mice per group. Original magnification, ×4.

vasopermeability in mice in which the hepatic portal circulation had been surgically ligated (**Fig. 4c**). Although that intervention might be expected to result in substantial alteration of the circulation in the mouse, potentially complicating interpretation of the results, an intravenously injected fluorescent probe did reach the distal extremities as in normal mice.

A function for vasoactive amines

Given the extremely rapid kinetic properties of the vascular leak induced by K/BxN serum, we sought an influence of vasoactive mediators. Histamine and serotonin are preformed vasoactive amines present in several cell types, particularly in mast cells, which can release them very quickly after activation. Both mediators induce rapid post-capillary venule permeability. Pharmacological blockade of the histamine H1 receptor with pyrilamine substantially decreased the rate of vascular leak, whereas treatment with the H2 receptor antagonist cimetidine or the combined H3 and H4 receptor antagonist thioperamide did not (**Fig. 5a**). The combination of pyrilamine and cimetidine was no more effective than was pyrilamine alone. Cyproheptadine blocks both the histamine and serotonin receptors and, as expected, resulted in a substantial reduction in the rate of serum-induced vasopermeability (**Fig. 5b**). Treatment with the more specific agent ketanserin, a serotonin 5-HT2A receptor blocker, also reduced macromolecular leakage (**Fig. 5b**). Leukotrienes are another class of mediators with potentially important influences on vascular permeability[18]. However, neither the production of newly formed leukotriene mediators via the 5-lipoxygenase pathway nor the leukotriene B_4 receptor BLT1 (data not shown) was required for vascular leak subsequent to the injection of arthritogenic serum. The relevance of systemic vasoactive amines to the K/BxN serum-transfer system was emphasized by the observation that plasma histamine concentrations increased in the first 5 min after serum injection (**Fig. 5c** and discussed below). This increase in plasma histamine concentration was not affected by ligation of the portal circulation (**Supplementary Fig. 3** online).

Histamine and serotonin were necessary for 'serum-promoted' vascular leak, but were they sufficient? Intravenous administration of each of these mediators was capable of rapidly inducing vasopermeability in the hindpaw (**Fig. 6**). Systemic injection of histamine was able to bypass the requirements for mast cells, neutrophils and FcRγ (**Fig. 6a**), placing its influence 'downstream' of or parallel to these required elements. Most unexpectedly, the increased vasopermeability induced by systemic administration of histamine or serotonin had limited tissue distribution, just like the macromolecular leakage occurring after injection of arthritogenic serum; that is, it was mainly in the distal extremities (**Fig. 6b**) and less commonly in the ears. As expected, administration of vasoactive amines alone in the absence of arthritogenic GPI-specific antibodies did not induce arthritis (data not shown). Although we have determined the simplest scenario incorporating these data (**Supplementary Fig. 4** online), there are some issues remaining, and other, more complex, scenarios can be envisioned.

DISCUSSION

Since the original description of the K/BxN mouse model of arthritis[1], the basis of the distal joint localization of its autoimmune inflammatory manifestations has remained an enigma. The fact that the initiation- and effector-phase triggers are systemic in nature was established by the identification of GPI, a ubiquitous enzyme in the glycolytic pathway, as the antigen recognized by both KRN T cells and the arthritogenic antibodies in K/BxN serum[4]. Part of the answer seems to lie in the specifics of articular structure[7,12]. Beyond residing in the cytoplasm of all cells and circulating at low concentrations in the blood, GPI is constitutively (in normal mice) aligned along the acellular surface where the cartilage borders the articular cavity, and immune complexes of GPI and antibody to GPI (anti-GPI) concentrate at these sites as arthritis develops[12]. Consisting almost entirely of IgG1-isotype antibodies, such immune complexes are potentially potent activators of the alternative pathway of complement, but in most locales such activity is kept in check by a repertoire of soluble and cellular complement inhibitors. In the joint, however, complement activation seems to run rampant, probably because of the absence of cellular inhibitors, such as decay-accelerating factor and CD59, at this acellular surface[12].

Another piece of the puzzle has been obtained from imaging studies[19,20]. A combination of rodent-scale positron emission tomography and biodistribution analyses has demonstrated that GPI-specific antibodies localize preferentially to the distal aspects of the front and rear limbs within minutes of intravenous injection into mice, reaching saturation by 20 min and remaining for at least 24 h (ref. 19). Collagen type II–specific antibodies alone do not show this rapid accumulation at articular sites, probably because unlike GPI,

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collagen type II does not circulate in the blood and thus circulating immune complexes cannot form. Indeed, coinjection of nonspecific immune complexes promotes the rapid accumulation of collagen type II–specific antibodies in the distal joints. More unexpectedly, nonspecific immune complexes also facilitate joint-specific localization of antibodies to nonjoint antigens, although they are not retained long-term[20]. The basis of the articular selectivity of the immune complex–facilitated rapid antibody access has not been identified. Our finding that nonspecific immune complexes elicited anatomically restricted vasopermeability is consistent with those published reports[19,20]. However, nonspecific immune complexes did not promote arthritis development in either case, indicating that simple access to the joint is not pathogenic. There must be longer-term residence of the immune complexes due to the targeting of some joint structure, eventually resulting in the mobilization of inflammatory effector mechanisms.

We have provided evidence here that that one or more radioresistant FcRγ-expressing cell type(s) may serve as the immune complex sensor to initiate the process of vascular leak. Precise identification of these cell types and where they are located remain undetermined. The loss of the vascular response after surgical ligation of the hepatic portal circulation suggests that an FcRγ-expressing sensor might reside in the liver. Indeed, FcγRIII is expressed by both Kupffer and sinusoidal endothelial cells of rodent liver[21], and the liver is the main organ for the uptake of IgG-containing immune complexes from the circulation[17], consistent with the tenfold higher accumulation of IgG in the liver (relative to that in other solid organs and the limbs) that has been reported[19]. It nevertheless remains possible that an organ other than the spleen that is drained by the portal circulation (such as the gut) contains an FcRγ-expressing cellular sensor. The finding that systemic histamine concentrations increased after serum administration in mice with ligation of the portal circulation raises the possibility that the liver (or gut) acts in parallel with the systemic histamine effect and that both inputs are required for immune complex–initiated vascular leak.

The earlier model[20] led us to speculate that neutrophils and mast cells would be activated by engagement of immune complexes by their cell surface FcγRIII to produce vascular leak. However, our results indicate that expression of FcRγ by neutrophils is not required. The situation for mast cells is more difficult to interpret, as some mast cell populations are radiosensitive and others are radioresistant; furthermore, engraftment of mast cell populations after irradiation and bone marrow transplantation proceeds at different rates in different tissues[22]. Thus, it is likely that in the bone marrow–chimeric mice we studied, a mixture of $FcR\gamma^+$ and $FcR\gamma^-$ mast cells existed and the $FcR\gamma^+$ mast cells may serve as immune complex sensors, releasing histamine and serotonin[23]. That interpretation is consistent with the observed increase in systemic histamine concentrations in mice with ligation of the portal circulation.

The observations described above further suggest that immune complexes activate neutrophils (and possibly mast cells) by a non-FcRγ-dependent mechanism to precipitate vascular leak. Notably, it has been reported that mast cell degranulation, plasma extravasation and neutrophil infiltration induced by intraplantar injection of complete Freund's adjuvant into mice depends on the neuropeptide neuromedin U, a proinflammatory compound produced mainly in the gut, central nervous system and hindpaws[24]. Thus, neuromedin U or compounds that act in a similar way are candidate molecules for the activation of innate immune cells by a non-FcRγ-dependent mechanism.

Exploiting a panel of pharmacological inhibitors, we have demonstrated here an important function for histamine and serotonin in immune complex–induced vascular leak. These vasoactive amines are well known to cause the formation of large gaps between endothelial cells, particularly in post-capillary venules[25]. Notably, systemic administration of either of these compounds recapitulated the anatomic restriction of vascular leak induced by immune complexes. Although many vascular beds may respond to vasoactive amines with leakage of small molecules such as dyes, the capacity for leakage of macromolecules as large as immune complexes, as shown here and before[20], seems to be restricted to certain vascular beds, particularly those of the distal extremities. Moreover, intravenous injection of smaller-molecule dyes (such as monastral blue B) can lead to leakage 'preferentially' around the paws of rodents[26]. In contrast, the localized vasopermeability described here is not simply due to passive extravasation through inherently porous endothelia, as the probe we used remains in the vasculature until triggering by immune complexes occurs. We therefore hypothesize that the machinery required for the rapid induction of macromolecular vasopermeability in response to systemically circulating vasoactive amines is limited to certain anatomical sites. Possible explanations for heightened sensitivity include higher expression of vasoactive amine receptors, unique receptor isoforms or signaling molecules 'downstream', differential patterns of innervation (as the vasoactive amines also serve as neurotransmitters) or even differences in the size of endothelial pores in resistant versus susceptible vascular beds[27].

The vascular response induced by K/BxN serum shares some but not all of the features of other well defined immune complex–initiated responses, such as the Arthus reaction and IgG-mediated systemic anaphylaxis. In the Arthus reaction, local formation of IgG-containing immune complexes activates a cascade of effector elements, including mast cells, neutrophils, FcγRIII, complement component C3, C5a and its receptor, and cytokines such as tumor necrosis factor and interleukin 1 (refs. 28–30). Increased vasopermeability is a hallmark of the early phase of the Arthus reaction[31] and is mediated by vasoactive amines released by mast cells[32]. In contrast, systemic anaphylaxis may be triggered by either IgE or IgG, although fatal reactions in rodents seem to be mediated mainly by IgG1 and FcγRIII (ref. 33). In contrast to IgE-mediated systemic anaphylaxis in which FcεRI-expressing mast cells are the key effectors, IgG-mediated anaphylaxis relies more on FcγRIII-expressing macrophages[34,35]. Like the Arthus reaction and IgG-mediated systemic anaphylaxis, the vascular response we have described here was triggered by IgG. However, its lack of dependence on complement distinguishes it from the Arthus reaction, and its reliance on mast cells, histamine and serotonin render it distinct from the present models of IgG-dependent systemic anaphylaxis[35]. Involvement of vasoactive amines, particularly serotonin, in promoting localized inflammatory responses has also been demonstrated for T cell–dependent contact sensitivity, in which platelets rather than mast cells seem to be a chief source of serotonin[36]. If platelets are involved in the vascular response we have reported here, our results suggest that they are triggered by a non-FcRγ-dependent mechanism.

How do these studies inform the present view of the genesis of arthritis? First, increased vascular permeability is not sufficient to induce arthritis. Many of the knockout mice tested here (deficient in C3, C5aR, interleukin 1 receptor, tumor necrosis factor and the neonatal Fc receptor) showed typical vascular leak after being injected with K/BxN serum but develop attenuated (or even no) arthritis[7,8,16]. These molecules must therefore be recruited and act at a later step in the disease process. Similarly, vascular leakage itself does not produce arthritis; arthritogenic autoantibodies are also necessary. Furthermore, although mast cells, neutrophils, FcRγ and FcγRIII were required for the early vascular permeability after serum transfer, our findings by no means preclude critical functions for these cells and receptors later

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during arthritis progression. A conceptually similar model of preferential targeting of autoantibodies exists in which systemic administration of lipopolysaccharide allows anti-neuronal antibodies to access the hippocampus in mice[3]. These findings collectively suggest that the pathogenicity of autoantibodies depends not only on their antigenic specificity but also on their capacity to reach target tissues. Thus, interventions designed to minimize vascular leak during early phases of immune complex–associated conditions, or perhaps during 'flare-ups' of disease, may prove therapeutically beneficial.

We postulate that the immune complex–triggered, vasoactive amine–dependent vascular leak demonstrated here is an early common pathway in arthritis elicited by immune complexes. This antigen-nonspecific response may allow enhanced access of immune complexes to the synovial space, leading to rapid recruitment of inflammatory cells and mediators and acceleration of arthritis progression. An amplifying function for vasoactive amines has been demonstrated in animal models of arthritis[37], but without establishment of their point of effect or recognition of their 'joint-preferential' nature. In addition, a function for vasoactive amines in patients with serum sickness is suggested by the efficacy of antihistamines in treating the associated arthralgia or arthritis. For transient arthritides (such as the passive transfer of K/BxN serum or antibodies to collagen type II in mice, or serum sickness in humans), eventual clearance of the immune complexes leads to resolution of the arthritis. In contrast, in a person in whom autoantibody formation is perpetuated because of an ongoing autoimmune response (such as humans with rheumatoid arthritis or systemic lupus erythematosus, or K/BxN T cell receptor–transgenic mice), progressive joint inflammation may ensue, especially if the autoantibodies remain bound to the joint surface[12].

Finally, our observation of joint-localized vasopermeability, in concert with published reports of joint-specific accumulation of circulating antibodies[19,20] and 'joint-preferential' activation of the alternative pathway of complement by immune complexes[12], draw attention to alternative mechanisms for achieving organ-specific autoimmune disease and emphasize the articulations as frequent targets of autoimmune pathology. Indeed, many mutant and knockout mouse strains develop arthritis, often unexpectedly[38–41]. In addition, arthritis is a secondary manifestation in a great variety of human infections and autoimmune disorders, such as hepatitis virus–associated and parvovirus B19–associated arthritides; Lyme disease; post-streptococcal arthritis and the related acute rheumatic fever; reactive arthritis; and systemic lupus erythematosus. We postulate that unique vascular characteristics underlie the vulnerability of the joints to autoimmune attack.

METHODS

Reagents. Chemicals were purchased from Sigma-Aldrich unless noted otherwise. Normal mouse IgG came from Jackson ImmunoResearch Laboratories, and the vascular probe AngioSense 680 was from VisEn Medical. IgG subtype–specific antibodies derived from hybridoma clones MOPC31C (IgG1), UPC10 (IgG2a), MOPC141 (IgG2b), and Y5606 (IgG3) were also from Sigma.

Mice. K/BxN T cell receptor–transgenic mice have been described[1] and were maintained at the animal facility of the Harvard School of Public Health (Boston, Massachusetts) for serum production. B6 mice, CD45.1 congenic B6 mice, BALB/c mice, mice devoid of FcγRIII, mice devoid of interleukin 1 receptor α-chain, mice deficient in tumor necrosis factor, mice lacking mast cells (W/W^v) and WBB6 mice were purchased from the Jackson Laboratory. Mice devoid of FcRγ[42] were purchased from Taconic and have been backcrossed 12 generations onto the B6 background. Mice devoid of C3 were a gift from M. Carroll (CBR Institute of Biomedical Research, Boston, Massachusetts); mice devoid of the C5a receptor were from C. Gerard (Children's Hospital Boston, Boston, Massachusetts); mice devoid of the neonatal Fc receptor[43] were from D. Roopenian (Jackson Laboratory); mice lacking FcεRIα, on a BALB/c background[44], were from J.-P. Kinet (Beth Israel Deaconess Medical Center); mice deficient in arachidonate 5-lipoxygenase[45] were from B. Kohler (University of North Carolina); mice deficient in leukotriene B_4 receptor 1 (ref. 46) were from A. Luster (Massachusetts General Hospital, Charlestown, Massachusetts); and neutrophil-deficient mice[47] were from S. Orkin (Children's Hospital Boston, Boston, Massachusetts). Wild-type littermates were used as controls. Mice used in experiments were 5–8 weeks of age unless noted otherwise.

Arthritis scoring. As described before[10], arthritis severity was assigned a score on a scale of 0–3 for each paw, resulting in a maximum score of 12 for an individual mouse. Ankle thickness was measured with a hand-held rotary caliper as described[5].

Stimulation of cultured mast cells. Bone marrow–derived mast cells were cultured from femoral flushes as described[10]. Mature bone marrow–derived mast cells (3×10^5 cells in 200 μl media) were added to flat-bottomed 96-well plates that had been coated overnight at 4 °C with purified mouse anti-trinitrophenol IgE (BD Pharmingen) in 0.1 M carbonate buffer, pH 9.5, at various concentrations. After brief centrifugation to initiate contact with plate-bound IgE, the bone marrow–derived mast cells were incubated for 6 h followed by collection of supernatant. The degranulation marker β-hexosaminidase was assessed as described[48].

Histology. Synovial mast cells were identified by toluidine blue staining of paraffin sections as described[10].

Bone marrow chimeras. Bone marrow was collected from donor mice using standard procedures. The 6-week-old recipient mice were irradiated with 1,000 rads and, 4 h later, were injected intravenously with 2×10^6 to 3×10^6 donor bone marrow cells. Before intravital microscopy or arthritis induction, successful bone marrow engraftment was confirmed by identification of the congenic marker CD45.1 (fluorescein isothiocyanate–conjugated A20; BD Pharmingen) or CD45.2 (allophycocyanin-conjugated clone 104; eBioscience) on peripheral blood leukocytes by flow cytometry. After induction of arthritis, sustained bone marrow engraftment was confirmed by analysis of peritoneal macrophages by flow cytometry, as described above.

Intravital confocal microscopy and quantification. A multichannel upright Radiance 2100 confocal microscope (Bio-Rad) based on a Nikon Eclipse upright microscope (Nikon) and with a custom-designed stage for intravital observations was used for intravital confocal microscopy. The stage was equipped with a translucent plate that could be regulated by a thermostat (37 °C) and Fluothane gas anesthesia. Anaesthetized mice were immobilized in the supine position on the heated stage. Images were acquired with Lasersharp imaging software (Bio-Rad). The red laser diode (637 nm) was used for excitation, and images were acquired through a 660-nm long-pass filter with a 4× dry objective. Unless noted otherwise, the vascular probe (100 μl/mouse) was administered intravenously to the anaesthetized mouse immediately before the mouse was transferred to the microscope stage. Single-plane 256- × 256-pixel confocal micrographs were obtained through intact skin at a depth of approximately 50–100 μm. Obtaining images every 3 s permitted reconstruction of the series into a time-lapse movie, allowing assessment of vascular leak over time. Quantification was done with Amira image analysis software (TGS) by calculation of the mean fluorescence intensity of each field of view; the maximum mean intensity per field of view was 256. Further analysis of the data, including calculation of the maximum rate of leak and duration at half-maximum rate, was done with a software program written in our Joslin laboratory (Boston, Massachusetts) using S-Plus (Insightful).

***In vitro* immune complex formation.** The formation of nonspecific immune complexes was accomplished by heating of normal mouse IgG or purified myeloma proteins of each mouse IgG subclass (0.5 mg/ml in PBS) at 65 °C for 25 min, followed by cooling to 4 °C. Complex formation was verified by enzyme-linked immunosorbent assay with plate-bound human C1q (Advanced

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Research Technology), coated at a concentration of 10 μg/ml in PBS, and standard alkaline phosphatase detection techniques.

Stimulation of vascular leak and pharmacological inhibition. All compounds were administered intravenously. The total infused volume was less than 350 μl/mouse. Serum derived from K/BxN mice was administered at a dose of 8 ml/kg body weight with a maximum of 200 μl/mouse. Normal mouse serum was obtained from nontransgenic BxN littermates and was given at a dose of 200 μl/mouse. IgG isolated from K/BxN serum by purification on a protein G column was given at a dose of 1 mg/mouse in a volume of 200 μl. The unbound fraction from the protein G purification was given at a dose of 200 μl/mouse. Purified normal mouse IgG or heat-aggregated immune complexes were administered at a dose of 100 μg/mouse in a volume of 200 μl.

Vasoactive amine inhibitors were dissolved in normal saline and were administered 15 min before serum injection at the following doses (mg reagent/kg body weight): pyrilamine maleate, 20 mg/kg; cimetidine, 30 mg/kg; thioperamide maleate, 30 mg/kg; cyproheptadine hydrochloride, 15 mg/kg; and ketanserin, 10 mg/kg. Histamine dihydrochloride was administered at a dose of 20 mg/kg and 5-hydroxytryptamine hydrochloride (serotonin) was administered at a dose of 10 mg/kg.

Determination of plasma histamine concentration. K/BxN serum or normal saline (200 μl mouse) was administered intravenously to B6 mice. Then, 5 min after treatment, mice were killed by CO_2 inhalation and blood was collected into EDTA-coated microtubes by retro-orbital bleeding. Samples in which obvious hemolysis occurred during blood collection were excluded to avoid an artifactual increase in the histamine concentration due to platelet lysis. After being centrifuged, the plasma was collected and was assayed for histamine concentration with a commercially available enzyme-linked immunosorbent assay kit (Research Diagnostics).

Surgical procedures. 'Skin windows' were surgically implanted into the backs of mice as described[49]. At least 1 week elapsed between implantation of the 'windows' and vascular imaging. To permit visualization of the cecum, laparotomies were done on anaesthetized mice, and the cecum was externalized and immobilized. Surface vessels were visualized by confocal microscopy. Splenectomy was done by open laparotomy and surgical cauterization. Ligation of the hepatic pedicle (including portal vein and hepatic artery) was done with a microsurgical clamp. Surgically manipulated mice were killed immediately after imaging. All procedures were approved by the Animal Care and Use Committee of Harvard Medical School (Boston, Massachusetts) and/or Massachusetts General Hospital (Charlestown, Massachusetts).

Note: Supplementary information is available on the Nature Immunology website.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Immunology* website for details).

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1. Kouskoff, V. *et al.* Organ-specific disease provoked by systemic autoreactivity. *Cell* **87**, 811–822 (1996).
2. Oono, T. *et al.* Organ-specific autoimmunity in mice whose T cell repertoire is shaped by a single antigenic peptide. *J. Clin. Invest.* **108**, 1589–1596 (2001).
3. Kowal, C. *et al.* Cognition and immunity; antibody impairs memory. *Immunity* **21**, 179–188 (2004).
4. Matsumoto, I., Staub, A., Benoist, C. & Mathis, D. Arthritis provoked by linked T and B cell recognition of a glycolytic enzyme. *Science* **286**, 1732–1735 (1999).
5. Korganow, A.-S. *et al.* From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. *Immunity* **10**, 451–461 (1999).
6. Maccioni, M. *et al.* Arthritogenic monoclonal antibodies from K/BxN mice. *J. Exp. Med.* **195**, 1071–1077 (2002).
7. Ji, H. *et al.* Arthritis critically dependent on innate immune system players. *Immunity* **16**, 157–168 (2002).
8. Ji, H. *et al.* Critical roles for interleukin-1 and tumor necrosis factor-α in antibody-induced arthritis. *J. Exp. Med.* **196**, 77–85 (2002).
9. Wipke, B.T. & Allen, P.M. Essential role of neutrophils in the initiation and progression of a murine model of rheumatoid arthritis. *J. Immunol.* **167**, 1601–1608 (2001).
10. Lee, D.M. *et al.* Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science* **297**, 1689–1692 (2002).
11. Corr, M. & Crain, B. The role of FcγR signaling in the K/B x N serum transfer model of arthritis. *J. Immunol.* **169**, 6604–6609 (2002).
12. Matsumoto, I. *et al.* How antibodies to a ubiquitous cytoplasmic enzyme may provoke joint-specific autoimmune disease. *Nat. Immunol.* **3**, 360–365 (2002).
13. Mangialaio, S., Staub, A., Benoist, C. & Mathis, D. The arthritogenic T cell receptor and its ligand in a spontaneous model of arthritis. *Arthritis Rheum.* **42**, 2517–2523 (1999).
14. Pober, J.S. & Cotran, R.S. The role of endothelial cells in inflammation. *Transplantation* **50**, 537–544 (1990).
15. Weissleder, R., Bogdanov, A., Tung, C.H., Jr. & Weinmann, H.J. Size optimization of synthetic graft copolymers for *in vivo* angiogenesis imaging. *Bioconjug. Chem.* **12**, 213–219 (2001).
16. Akilesh, S. *et al.* The MHC class I-like Fc receptor promotes humorally mediated autoimmune disease. *J. Clin. Invest.* **113**, 1328–1333 (2004).
17. Lovdal, T., Andersen, E., Brech, A. & Berg, T. Fc receptor mediated endocytosis of small soluble immunoglobulin G immune complexes in Kupffer and endothelial cells from rat liver. *J. Cell Sci.* **113**, 3255–3266 (2000).
18. Benjamim, C.F., Canetti, C., Cunha, F.Q., Kunkel, S.L. & Peters-Golden, M. Opposing and hierarchical roles of leukotrienes in local innate immune versus vascular responses in a model of sepsis. *J. Immunol.* **174**, 1616–1620 (2005).
19. Wipke, B.T., Wang, Z., Kim, J., McCarthy, T.J. & Allen, P.M. Dynamic visualization of a joint-specific autoimmune response through positron emission tomography. *Nat. Immunol.* **3**, 366–372 (2002).
20. Wipke, B.T., Wang, Z., Nagengast, W., Reichert, D.E. & Allen, P.M. Staging the initiation of autoantibody-induced arthritis: a critical role for immune complexes. *J. Immunol.* **172**, 7694–7702 (2004).
21. Lovdal, T. & Berg, T. Transcription of Fcγ receptors in different rat liver cells. *Cell Biol. Int.* **25**, 821–824 (2001).
22. Kitamura, Y., Shimada, M., Hatanaka, K. & Miyano, Y. Development of mast cells from grafted bone marrow cells in irradiated mice. *Nature* **268**, 442–443 (1977).
23. Theoharides, T.C., Bondy, P.K., Tsakalos, N.D. & Askenase, P.W. Differential release of serotonin and histamine from mast cells. *Nature* **297**, 229–231 (1982).
24. Moriyama, M. *et al.* The neuropeptide neuromedin U promotes inflammation by direct activation of mast cells. *J. Exp. Med.* **202**, 217–224 (2005).
25. Majno, G. & Palade, G.E. Studies on inflammation. 1. The effect of histamine and serotonin on vascular permeability: an electron microscopic study. *J. Biophys. Biochem. Cytol.* **11**, 571–605 (1961).
26. Majno, G. *et al.* A pancreatic venular defect in the BB/Wor rat. *Am. J. Pathol.* **128**, 210–215 (1987).
27. Knight, A.D. & Levick, J.R. Morphometry of the ultrastructure of the blood-joint barrier in the rabbit knee. *Q. J. Exp. Physiol.* **69**, 271–288 (1984).
28. Sylvestre, D.L. & Ravetech, J.V. A dominant role for mast cell Fc receptors in the Arthus reaction. *Immunity* **5**, 387–390 (1996).
29. Hopken, U.E., Lu, B., Gerard, N.P. & Gerard, C. Impaired inflammatory responses in the reverse Arthus reaction through genetic deletion of the C5a receptor. *J. Exp. Med.* **186**, 749–756 (1997).
30. Baumann, U. *et al.* Distinct tissue site-specific requirements of mast cells and complement components C3/C5a receptor in IgG immune complex-induced injury of skin and lung. *J. Immunol.* **167**, 1022–1027 (2001).
31. Hayashi, H., Yoshinaga, M., Koono, M., Miyoshi, H. & Matsumura, M. Endogeneous permeability factors and their inhibitors affecting vascular permeability in cutaneous Arthus reactions and thermal injury. *Br. J. Exp. Pathol.* **45**, 419–435 (1964).
32. Ramos, B.F., Zhang, Y., Angkachatchai, V. & Jakschik, B.A. Mast cell mediators regulate vascular permeability changes in Arthus reaction. *J. Pharmacol. Exp. Ther.* **262**, 559–565 (1992).
33. Miyajima, I. *et al.* Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and FcγRIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis. *J. Clin. Invest.* **99**, 901–914 (1997).
34. Strait, R.T., Morris, S.C., Yang, M., Qu, X.W. & Finkelman, F.D. Pathways of anaphylaxis in the mouse. *J. Allergy Clin. Immunol.* **109**, 658–668 (2002).
35. Finkelman, F.D., Rothenberg, M.E., Brandt, E.B., Morris, S.C. & Strait, R.T. Molecular mechanisms of anaphylaxis: lessons from studies with murine models. *J. Allergy Clin. Immunol.* **115**, 449–457 (2005).
36. Geba, G.P. *et al.* Delayed-type hypersensitivity in mast cell-deficient mice: dependence on platelets for expression of contact sensitivity. *J. Immunol.* **157**, 557–565 (1996).
37. Harbuz, M.S. *et al.* The role of endogenous serotonin in adjuvant-induced arthritis in the rat. *Br. J. Rheumatol.* **35**, 112–116 (1996).

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38. Yu, P. *et al.* Autoimmunity and inflammation due to a gain-of-function mutation in phospholipase Cγ2 that specifically increases external Ca^{2+} entry. *Immunity* **22**, 451–465 (2005).
39. Horai, R. *et al.* Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *J. Exp. Med.* **191**, 313–320 (2000).
40. Hang, L., Theofilopoulos, A.N. & Dixon, F.J. A spontaneous rheumatoid arthritis-like disease in MRL/l mice. *J. Exp. Med.* **155**, 1690–1701 (1982).
41. Sakaguchi, N. *et al.* Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. *Nature* **426**, 454–460 (2003).
42. Takai, T., Li, M., Sylvestre, D., Clynes, R. & Ravetch, J.V. FcRγ chain deletion results in pleitrophic effector cell defects. *Cell* **76**, 519–529 (1994).
43. Roopenian, D.C. *et al.* The MHC class I-like IgG receptor controls perinatal IgG transport, IgG homeostasis, and fate of IgG-Fc-coupled drugs. *J. Immunol.* **170**, 3528–3533 (2003).
44. Dombrowicz, D., Flamand, V., Brigman, K.K., Koller, B.H. & Kinet, J.P. Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor α chain gene. *Cell* **75**, 969–976 (1993).
45. Goulet, J.L., Snouwaert, J.N., Latour, A.M., Coffman, T.M. & Koller, B.H. Altered inflammatory responses in leukotriene-deficient mice. *Proc. Natl. Acad. Sci. USA* **91**, 12852–12856 (1994).
46. Tager, A.M. *et al.* BLTR mediates leukotriene B_4-induced chemotaxis and adhesion and plays a dominant role in eosinophil accumulation in a murine model of peritonitis. *J. Exp. Med.* **192**, 439–446 (2000).
47. Hock, H. *et al.* Intrinsic requirement for zinc finger transcription factor Gfi-1 in neutrophil differentiation. *Immunity* **18**, 109–120 (2003).
48. Katz, H.R. *et al.* Secretory granule mediator release and generation of oxidative metabolites of arachidonic acid via Fc-IgG receptor bridging in mouse mast cells. *J. Immunol.* **148**, 868–871 (1992).
49. Huang, Q. *et al.* Noninvasive visualization of tumors in rodent dorsal skin window chambers. *Nat. Biotechnol.* **17**, 1033–1035 (1999).

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