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Bone Marrow-Derived Mast Cells Accumulate in the Central Nervous System During Inflammation but Are Dispensable for Experimental Autoimmune Encephalomyelitis Pathogenesis

Jami L. Bennett,* Marie-Renée Blanchet,† Linlin Zhao,‡ Lori Zbytnuik,* Frann Antignano,‡ Matthew Gold,* Paul Kubes,† and Kelly M. McNagny*‡

Reports showing that W/Wv mice are protected from experimental autoimmune encephalomyelitis (EAE, a murine model of multiple sclerosis), have implicated mast cells as an essential component in disease susceptibility, but the role of mast cell trafficking has not been addressed. In this study, we have used both mast cell transplantation and genetic mutations (Cd34−/−, W/Wv, Wsh/Wsh) to investigate the role of mast cell trafficking in EAE in detail. We show, for the first time, that bone marrow-derived mast cells are actively recruited to the CNS during EAE. Unexpectedly, however, we found that EAE develops unabated in two independent genetic backgrounds in the complete absence of mast cells or bone marrow-derived mast cell reconstitution. We conclude that although mast cells do accumulate in the brain and CNS during demyelinating disease via peripheral mast cell trafficking, they are completely dispensable for development of disease. The Journal of Immunology, 2009, 182: 5507–5514.

M ast cells are classically known for their role in Th2-type immune responses including allergic inflammation, asthma, and anaphylaxis. Thus, mature tissue localized mast cells (decorated with high affinity Fc receptor-bound IgE and IgG), respond to mucosal allergen exposure via degranulation and release of inflammatory mediators (1). Recent reports have implicated mast cells in an ever-widening array of normal and pathological responses. These include such diverse processes as: allograft tolerance (2), autoimmune arthritis and allergic asthma (3–4), inflammatory bowel disease (5), tissue remodeling and fibrosis (6), VEGF-mediated angiogenesis in tissue repair or during epithelial cell carcinogenesis (7–8), TNF-mediated innate immune clearance of bacteria and lymph node hypertrophy (9), and protease-mediated protection from morbidity and mortality associated with envenomation (10).

Implication of mast cells in these responses has been elucidated through the development of invaluable mouse strains with genetic lesions rendering them deficient in mast cell formation or function. Two widely used strains are W/Wv and Wsh/Wsh mice (11, 12). The W/Wv mouse is an F1 hybrid bearing two mutations (a point chromosome inversion in transcriptional regulatory elements of c-kit, has gained popularity as it is mast cell-deficient but lacks the anemia and other nonhematopoietic defects of the W/Wv mice and can be maintained as a homozygous line on a C57BL/6 background. Each strain can be reconstituted with wild-type (WT) or mutant bone marrow-derived mast cells (BMMC). Thus, W/Wv and Wsh/Wsh mice offer excellent tools to delineate mast cell-dependent processes. Indeed, this led to the discovery of most of their aforementioned new functions.

Recently we delineated an additional tool for mast cell function in the CD34-deficient mouse. CD34 is a stem cell surface sialoglycoprotein and we have shown this molecule is a relatively selective marker of mature mast cells in mice and is required for efficient trafficking in vivo (13). Thus, we have found that, although Cd34−/− mice have normal peripheral tissue mast cell numbers at steady-state, they have a profound defect in mast cell mobilization and recruitment during inflammation of mucosal sites (4, 14). Currently it is unclear whether mast cell-dependent disease requires local proliferation in situ or recruitment of mast cell precursors from the circulation. Therefore, these mice offer an unprecedented opportunity to study the role of mast cell trafficking in disease, and to further explore the importance of CD34 expression on mast cell accumulation in nonmucosal sites.

Experimental autoimmune encephalomyelitis (EAE) is a well-characterized murine model of multiple sclerosis used extensively to understand the role of specific molecules and cell subsets in disease pathology (15, 16). In recent studies it has been reported that mast cell deficient mice are resistant to myelin oligodendrocyte glycoprotein (MOG)35–55-induced EAE (17, 18). Reconstitution of mast cell deficient W/Wv animals with WT BMMC was...
The data suggested that mast cell-dependent effects were due to systemic mast cell activation because transplanted mast cells were observed peripherally, but not in the CNS during active multiple sclerosis (19). Mast cell-mediated effects were thought to be via both activating- and inhibitory-Fc receptors (18) controlling autoreactive T cell responses from outside the CNS (19, 20). The relative importance of mast cell trafficking and recruitment was not addressed in these reports, as it has been in other studies (14).

We hypothesized that if mast cell recruitment to the site of inflammation was required for demyelinating disease, \( \text{Cd34}^{-/-} \) mice would be protected from EAE due to decreased peripheral mobility of mast cells. Alternatively, if seeding or trafficking of CNS or perivascular mast cells during development were sufficient for their functional role in EAE, then \( \text{Cd34}^{-/-} \) mice would be susceptible to EAE. We further hypothesized that reconstitution of mast cell-deficient W/W\(^v\) or Wsh/Wsh mice with \( \text{Cd34}^{-/-} \) mast cells would not be sufficient to restore disease susceptibility due to previously documented migration defects in these cells (4, 14, 21).

In this study, we report that \( \text{Cd34}^{-/-} \) mice are indeed susceptible to MOG-induced disease, initially suggesting that mast cell trafficking is not a requirement for EAE pathogenesis. However, we reported to restore susceptibility to EAE induction. Intriguingly, the data suggested that mast cell-dependent effects were due to systemic mast cell activation because transplanted mast cells were observed peripherally, but not in the CNS during active multiple sclerosis (19). Mast cell-mediated effects were thought to be via both activating- and inhibitory-Fc receptors (18) controlling autoreactive T cell responses from outside the CNS (19, 20). The relative importance of mast cell trafficking and recruitment was not addressed in these reports, as it has been in other studies (14).

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observed that BMMC accumulate in the CNS during EAE, suggesting that mast cells are recruited in the course of CNS inflammation. Surprisingly (and in contrast to published literature), we also found that both W/Wv and Wsh/Wsh mice develop severe EAE, and reconstitution of these mice with either WT or Cd34−/− mast cells did not alter the course of MOG-induced disease. We conclude that neither mast cells nor mast cell trafficking play an essential role in this model of multiple sclerosis.

Materials and Methods

Mice

All mice were maintained at The Biomedical Research Centre under pathogen-free conditions, and all procedures involving mice were approved by the University of British Columbia Animal Care Committee. Cd34−/− mice (C57BL/6 background) were provided by Dr. T. W. Mak (Princess Margaret Hospital, University of Toronto, Toronto, Ontario, Canada) (22). Female C57BL/6, WB6F1/J-kitW/kitWv (W/Wv) and WB6F1/J-kitW-sh/HNihrJae (Wsh/Wsh) congenic littermate controls, and B6.Cq-KitW-sh/HNLRJae-Bsmj mice (Wsh/Wsh) mice were purchased from The Jackson Laboratory. Standard chow and water were provided ad libitum.

EAE induction

EAE was initiated using standard protocols and reagents. In brief, MOG35–55 peptide (MEVGWYRSPFSRVVHLYRNGK) was synthesized at The UBC Peptide Core Facility. Each recipient received 200 μg of peptide resuspended in 100 μl of PBS, emulsified with 100 μl CFA containing 8 mg/ml Mycobacterium tuberculosis H37RA (Difco). Mice were immunized on shaved hind flanks over three sites, followed by i.v. administration of 200 ng of pertussis toxin in PBS at days 0 and 2, allowing for reconstitution of mast cell compartments and EAE was initiated as described at least 6 wk after transplant.

Generation of BM chimeric mice

BM was flushed from dissected femurs of C57BL/6 or Cd34−/− mice with PBS under aseptic conditions. RBC were lysed using 0.1M NH4Cl, and the remaining cells were washed, counted, and resuspended in 200 μl PBS. Recipients received lethal irradiation, followed by i.v. transfer of 5 × 106 donor cells via the tail vein. All recipients were checked for chimera by examining CD45.1 and CD45.2 levels on peripheral blood cells. EAE was initiated as described at least 6 wk after transplant.

Mast cell reconstitution of W/Wv and Wsh/Wsh mice

BM was flushed from dissected femurs of C57BL/6 or Cd34−/− mice with PBS under aseptic conditions. RBC were lysed using 0.1M NH4Cl, and the remaining cells were washed and resuspended in complete RPMI 1640 containing 10% FBS, penicillin/streptomycin, sodium pyruvate, l-glutamine, and 16 U/ml IL-3 obtained from recombinant sources or WEHI-3B conditioned medium. Cells were transferred periodically to fresh flasks to remove adherent cells, and maintained for at least 4 wk to allow mast cell differentiation. For adoptive transfer, 105 cells were resuspended in 200 μl of PBS and injected i.v. into 4–6 wk old recipients. A period of 8 wk was allowed for reconstitution of mast cell compartments and EAE was initiated as described.

FACS analysis of inflammatory infiltrates in the spinal cord

Spinal cords were isolated from perfused animals by flushing the vertebral column with 2–5 ml PBS through a blunted 18-gauge needle. The tissue was minced in high salt HBSS (Life Technologies) containing 400 U/ml collagenase IV (Sigma-Aldrich), incubated for 30 min at 37°C, and cells separated by passage through 70-μm nylon mesh filter cups (BD Bioscience). Single cell suspensions were washed with HBSS and pelleted by centrifugation at 1200 rpm for 5 min at 4°C. Cells were then resuspended in 2–5 ml 30% Percoll solution (Amersham Pharmacia) and leukocytes isolated by centrifugation at 22°C, 1200 rpm (328 × g). Leukocytes were recovered from the pellet and washed with FACS buffer (PB: PBS containing 1% FBS, 2 mM EDTA, 0.05% sodium azide), and stored at 4°C for Ab staining and flow cytometric analysis. For examination of granulocyte
fractions, 70% Percoll was underlaid beneath the 30% Percoll layer before centrifugation, and both the 30/70 interface (lymphocytes/monocytes) and pellet (granulocyte) fractions were analyzed. Cells were washed in FB followed by incubation with specific Abs to mouse cKit, CD11b, CD4, CD8a, B220, CD45, or CD3 (BD Pharmingen) at a predetermined optimal concentration for at least 15 min on ice. Cells were then washed and re-suspended in 0.2 ml FB. Data collection was performed on a FACS Calibur or LSRII (BD Biosciences) flow cytometer using CellQuest or Diva software and analyzed using FlowJo software.

Histology
Histological evaluation was performed on tissue from representative mice from each experimental group. Mice were anesthetized with Avertin solution and perfused through the left cardiac ventricle with 40 ml of PBS. Spinal cords were recovered from the vertebral canal by either flushing with PBS using a 25-gauge needle or dissection. The lumbar spinal cord segment was fixed in 10% neutral buffered formalin and embedded in paraffin. Sections from each animal were stained with standard H&E. (WaxIt Histology Services, University of British Columbia). Alternatively, some animals were perfused with PBS followed by 30 ml of 2% paraformaldehyde (PFA) and the brain and spinal cord recovered by dissection. Tissues were postfixed for 48 h in 2% PFA, and then stored in 70% ethanol until processing. The tissue was embedded in paraffin, sectioned at 5 μm, and stained with acidified tolue blue or chloroacetate esterase to identify mast cells. Representative photomicrographs were taken using a Zeiss microscope equipped with a digital camera. Images were created using Openlab and Adobe Illustrator software.

Ag recall proliferation
Proliferative capacity of CD4+ T cells isolated from C57BL/6, Cd34+/−, and WSh/Wsh animals was assayed by conventional mitogenic stimulation. Spleen and lymph node tissue was recovered from EAE-induced animals at day 21 postimmunization. A single cell suspension of 5 × 10^6 bulk spleen or lymph node cells were plated in 200 μl in triplicate wells, in the presence of 0, 0.5, 5, or 50 μM MOG35–55 Ag in DMEM containing 5% FBS, 1 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 μM nonessential amino acids, and 5 × 10^−5 M 2-ME (complete DMEM-5, all components from Invitrogen) for culture. Cultures were incubated at 37°C, 5% CO2 for 72 h. Wells were harvested onto filters and counted using an LKB Betaplate Harvester and Liquid Scintillation Counter (LKB Wallac). Δcpm was calculated by subtracting average cpm values of unstimulated wells for each group.
Cytokine analysis

Cytokine levels were analyzed in both serum samples and Ag recall supernatant. Blood was collected by cardiac puncture from anesthetized animals at the time of harvest, immediately before perfusion. Approximately 400 μl of blood was stored at room temperature for 2 h and the serum separated by centrifugation at 4000 × g for 20 min. Serum was transferred to fresh tubes and stored at −20°C until analysis. To analyze the cytokine concentration in serum samples, the FACS bead-based CBA Mouse Inflammation kit (BD Pharmingen, Mouse Inflammation Kit) was used according to the manufacturer’s instructions. For Ag recall responses, spleen and lymph node tissue was recovered from EAE-induced animals at day 21 postimmunization. A single cell suspension of 2.5 × 10^6 bulk spleen or lymph node cells were plated in 24-well plates in the presence of 0, 0.5, 5, or 50 μM MOG35–55 Ag in DMEM containing 5% FCS, 1 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 μM nonessential amino acids, and 5 × 10^-5 M 2-ME (complete DMEM-5, all components from Invitrogen) for culture. Cultures were incubated at 37°C, 5% CO₂ for 48 h. Supernatants were collected and analyzed immediately with the FACS bead-based CBA Mouse Inflammation and Th1/Th2 kit (BD Pharmingen, Mouse Inflammation Kit) according to the manufacturer’s instructions.

Results

Cd34^-/- mice are fully susceptible to clinical disease following EAE induction

Previously, we published that while Cd34^-/- mice have a normal frequency of peripheral mast cells at steady state, CD34 is essential for efficient trafficking of mast cells and their precursors to peripheral tissues in response to inflammatory challenge (4, 14, 21). To test the role of CD34 and mast cell recruitment in susceptibility to EAE, 6–8 wk old Cd34^-/- mice (22) and C57BL/6 controls were immunized with encephalitogenic MOG35–55 peptide and monitored for clinical disease development (Fig. 1A). In five independent experiments, we found similar disease onset and incidence in Cd34^-/- and control groups. In two of these experiments, no differences were observed in severity of disease (data not shown), while in three experiments we observed exacerbated clinical symptoms in the Cd34^-/- mice at later time-points in disease (Fig. 1A) *p < 0.05.

Activated microglia/macrophage and mast cell accumulation in the CNS of Cd34^-/- mice is increased compared with C57BL/6 controls

Because Cd34^-/- mice exhibit normal numbers of resident mast cells at steady state, we reasoned that resident CNS or vascular mast cells could be sufficient for disease development in these mice. To test this hypothesis, we assessed the accumulation of inflammatory cells in Cd34^-/- and C57BL/6 control animals by flow cytometry. At day 21 postimmunization, brains and spinal cords were recovered and infiltrating cell subsets were analyzed by flow cytometry. As shown in Fig. 1, B and C, we did not observe a significant difference in either the percentage or the absolute numbers of CD4^+ or CD8^+ T cells, or B220^+ B cells in the CNS. However, activated microglia/macrophages/dendritic cells (CD45^/CD11b^+) and mast cell (CD45^/c-kit^) accumulation in the CNS of Cd34^-/- mice was consistently and significantly increased compared with controls, in terms of both percentage and absolute number of cells (*p < 0.05). The average total hematopoetic cell infiltrate in the CNS of C57BL/6 and Cd34^-/- groups was clearly increased in EAE tissue compared with naive CNS (Fig. 1D1) but was not significantly different between groups with EAE. Consistent with published work detailing CCL2-mediated macrophage accumulation and EAE development (23), we found increased levels of CCL2 in the serum of Cd34^-/- mice (Fig. 1DII), providing an explanation for increased macrophages and possibly increased disease severity in Cd34^-/- mice. H&E staining of spinal cord histological sections (Fig. 2A) revealed similar focal inflammatory cell infiltration in C57BL/6 and Cd34^-/- mice. We conclude that Cd34^-/- mice are fully susceptible to EAE induction and exhibit equal or greater severity of disease compared with C57BL/6 controls.


*Mast cell accumulation is not required for EAE*

*Cd34<sup>/−</sup> mice generate similar proliferative and inflammatory cytokine responses to the I<sup>κ</sup>B myelin epitope MOG<sub>35-55</sub>*

At the peak of acute EAE, we collected spleen and lymph node tissue from individual animals in each group with similar average clinical scores and analyzed the ability of Cd34<sup>/−</sup> cells to generate recall immune responses to MOG. In Fig. 2B we show similar proliferative potential in Cd34<sup>/−</sup> cells compared with controls. There was a trend toward decreased total IFN-γ and TNF-α production (Fig. 2C), although these differences were not statistically significant. IL-2 production, however, was significantly decreased in Cd34<sup>/−</sup> cells compared with WT (*, p < 0.05) in three separate experiments.

*Mast cells accumulating in the CNS during EAE are donor bone marrow-derived*

Few studies have examined the role of mast cell precursors and migration in neurodegenerative disease. Although we anticipated a defect in migration of Cd34<sup>/−</sup> mast cells based on previous work in our laboratory (4, 14, 21, 24), this approach did not directly differentiate between locally proliferating mast cells and infiltrating peripheral mast cells. To address this question, we used bone marrow chimeras and flow cytometry to examine endogenous vs newly recruited mast cell accumulation in the CNS of mice with EAE. Bone marrow chimeric mice were prepared by i.v. infusion of either WT or Cd34<sup>/−</sup> bone marrow mast cell reconstitution were visually confirmed using chloroacetate esterase staining. Cells from individual spinal cords (W/W<sup>+</sup> and WT littermates n = 5 each; naive W/W<sup>+</sup> n = 2) at day 21–25 postimmunization were counted using a standard hemacytometer. Total infiltrating mononuclear cells in W/W<sup>+</sup> and littermate control animals are not significantly different. D. Spleen and lymph node C57BL/6 and Wsh/Wsh cells were cultured in the presence of varying concentrations of MOG<sub>35-55</sub> peptide to assess recall proliferation responses. Student’s t test was used to determine significance comparing average clinical scores or cell number. *, p < 0.05.

![Figure 5](http://www.jimmunol.org/)

**FIGURE 5.** Diffuse inflammatory infiltrate, mast cell localization, and intact proliferative responses in W/W<sup>+</sup> and Wsh/Wsh mice with EAE. A. H&E stained sections of representative CNS histological sections from W/W<sup>+</sup> and Wsh/Wsh, with or without WT or Cd34<sup>/−</sup> bone marrow mast cell reconstitution. (n = 4 each). B. Mast cells in the CNS of littermate controls and W/W<sup>+</sup> with WT or Cd34<sup>/−</sup> bone marrow mast cell reconstitution were visually confirmed using chloroacetate esterase staining. C. Cells from individual spinal cords (WT and WT littermates n = 5 each; naive W/W<sup>+</sup> n = 2) at day 21–25 postimmunization were counted using a standard hemacytometer. Total infiltrating mononuclear cells in W/W<sup>+</sup> and littermate control animals are not significantly different. D. Spleen and lymph node C57BL/6 and Wsh/Wsh cells were cultured in the presence of varying concentrations of MOG<sub>35-55</sub> peptide to assess recall proliferation responses. Student’s t test was used to determine significance comparing average clinical scores or cell number. *, p < 0.05.
cord tissue sections from EAE mice was used to visually confirm
the presence of mast cells in CNS tissue (Fig. 3C). Large bright red
cells were readily identified as mast cells in these sections. These
results support the novel conclusion that during EAE mast cells
develop, de novo, from bone marrow progenitors and infiltrate
the CNS.

Wsh/Wsh and W/Wv mice are fully susceptible to EAE induction
Wsh/Wsh and W/Wv have been used recently to compare and contrast observations
made in the well known mast cell deficient W/Wv strain. We also considered the Wsh/Wsh strain as a possible improvement to W/Wv strain due to the ability to maintain homozygous breeders on a fully C57BL/6, H-2b background (12). Previous studies using W/Wv mice reported a requirement for mast cells in clinical development of EAE through reconstitution studies, where restoration of disease was achieved after addition of BMMC via tail vein injection (17). Thus, we next examined the disease course of EAE in mast cell deficient Wsh/Wsh and also in W/Wv and Wsh/Wsh mice reconstituted with either WT or Cd34−/− BMMC. To generate mast cells, bone marrow of WT or Cd34−/− mice was cultured for 8 wk with IL-3 to induce terminal differentiation of BMMC. WT or Cd34−/− BMMC were then adoptively transferred into W/Wv and Wsh/Wsh mice. These mice were rested for a period of 8 wk to allow full reconstitution of mast cell compartments. Nonreconstituted W/Wv and Wsh/Wsh were analyzed in parallel as controls (12). Interestingly, we found that mice receiving either WT and Cd34−/− BMMC developed severe EAE after MOG immunization (Fig. 4A).

To determine whether animal age or laboratory variation were factors, two sets of experiments were performed. First, 8 wk old WBB6F1/J-kitWv/Wv (W/Wv) mice, WBB6F1/J-kitWv/Wv congenic littermates, and C57BL/6 positive control groups were immunized with MOG35-55 peptide and monitored for clinical disease development. The age of mice in these experiments was that used for standard EAE induction, without the additional 8 wk required for mast cell reconstitution. Surprisingly, we found that nonreconstituted 8-wk-old W/Wv and Wsh/Wsh mice also developed severe EAE (Fig. 4, BI and BII). In some cases, clinical disease observed in nonreconstituted W/Wv and Wsh/Wsh mice was sufficiently severe that the mice were moribund at the peak of disease and had to be euthanized. We did observe a trend toward decreased severity during onset and peak of disease in W/Wv mice; however, this group ultimately achieved similar severity of disease to WT littermate controls over the course of disease.

To rule out laboratory or animal housing/environmental variation, immunizations and disease courses were monitored at two independent universities and laboratories (University of British Columbia, British Columbia, Canada and University of Calgary, Alberta, Canada) using the same standard priming protocol. Again, in both laboratories we observed similar disease susceptibility in W/Wv mice (Fig. 4BIII). Thus, we conclude that susceptibility of mast cell deficient mice to EAE is independent of age, laboratory reagent variation, or animal housing status.

Accumulation of hematopoietic infiltrate is evident in W/Wv and Wsh/Wsh mice undergoing EAE
Infiltrate accumulation is evident in the architecture of tissue in mast cell deficient animals undergoing EAE. H&E histological preparations from either W/Wv or Wsh/Wsh spinal cords revealed extensive inflammatory cell infiltration (Fig. 5A). It is interesting to note the diffuse distribution of infiltrating cells in the mast cell deficient hosts. Although rare and located exclusively in the meningeal zones, mast cells were also identified in mast cell reconstituted W/Wv undergoing EAE using CAE staining (Fig. 5B). Total infiltrating hematopoietic populations were counted using a standard hemocytometer following the density centrifugation preparation of spinal cord tissue from naive and EAE-induced animals. Infiltration of mononuclear cells following EAE induction is evident by comparing infiltrate numbers in diseased vs naive tissue. As shown in Fig. 5C, we observed no statistically significant difference in average total infiltrate between W/Wv and littermate WT controls at day 25 following disease induction. Moreover, Wsh/Wsh mice showed a comparable or higher immune response to the MOG Ag in both spleen and lymph node in a proliferation assay (Fig. 5D). We conclude that neither mast cells, nor mast cell trafficking to the CNS, play an essential role in the development of EAE.

Discussion
Our data suggest that CD34 is not required for susceptibility to EAE and that CD34 loss may, in fact, exacerbate the disease course. Because CD34 is expressed by endothelial cells, microglia, mast cells, and eosinophils (13, 25–27), the loss of CD34 from any one of these cell types could account for the enhanced disease. We therefore focused our attention on whether CD34 expression on mast cells specifically modulates EAE severity.

Efforts to understand the role of CD34 specifically on mast cells in EAE were complicated in this study by the surprising observation that W/Wv and Wsh/Wsh mice were susceptible to disease. The susceptibility of mast cell-deficient mice to EAE despite previous publications to the contrary is perplexing, more so considering the varied haplotypes of the two strains. Although most reagents and all mice were obtained from the same sources as those described (14, 17–20), a number of more subtle factors could explain these discordant results. These include the age of the mice (reconstitution of mice for 8 wk with cultured mast cells necessitated the use of older animals), the concentration of adjuvant used, and potential exposure of animals to stress or pathogens during the disease course. Histological detection of mast cells in the context of inflammation has proven challenging, due to their sparse distribution and the reliance of metachromatic stains on intact granules. Previous reports of a failure to detect mast cells in the CNS is likely due to instead to an inability to locate granulated cells (28).

We found that neither age nor laboratory environment was a factor in our ability to induce disease in W/Wv mice in two independent experiments. It is well documented that stress levels (affecting steroid release) and pathogen exposure can have a profound effect on ameliorating clinical symptoms of both multiple sclerosis and EAE (29–31). Because of the pleiotropic roles of mast cells in a variety of biological functions, it is conceivable that mast cell deficient mice could be particularly sensitive to environmental variation. Thus, regardless of the cause for this discrepancy, our results using standard protocols for disease induction indicate that mast cells and their trafficking are clearly not required for disease.

An interesting and novel observation in this study is that modulation of mast cell levels in the CNS during inflammation (via absence of mast cells in W/Wv and Wsh/Wsh mice or an increase in mast cell accumulation in Cd34−/− mice) in the CNS does not appear to modulate EAE severity. Thus, it is unlikely that targeting mast cells for therapy would prevent disease development. Although our results suggest that mast cells are dispensable for development of disease, we do not discount the ability of mast cells to augment local tissue inflammation; this report is consistent with previous studies detecting mast cells associated with inflammatory infiltrate in both multiple sclerosis and EAE tissue, as well as products of activated mast cells in the cerebrospinal fluid of multiple sclerosis patients (32, 33). Specifically, disruption of the
blood brain barrier is well documented in these diseases and serum factors such as fibrinogen are reported to stimulate mast cells leading to degranulation (34). It is easy to conceive of a scenario in which the appropriate chemotactic stimulus is present in the context of inflamed CNS tissue whereby mast cells are recruited to the damage site, exposed to serum factors (including fibrinogen), and induced to degranulate, adding to the inflammatory milieu in the tissue and cerebrospinal fluid. Furthermore, mast cells are ample sources of cytokines such as TNF and IL-6 which likely contribute to the deleterious inflammatory cascades in the demyelinating CNS. It is known that mast cells produce a number of soluble factors that can play a major role in tissue remodeling, angiogenesis, and repair (VEGF, TGF, CCL2, etc.). A possible role for mast cells in the CNS could include stimulating revascularization and repair of damaged tissues after an autoimmune response. Indeed, despite their dispensability for disease induction and due to their appropriate localization, it may be possible to use BMMC as therapeutic delivery vehicles to sites of inflammation. Future studies should investigate the role of mast cells in inflammatory diseases as a network, which requires a critical examination of specific tissue localization, function, and dynamic interaction with endogenous cells.

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Disclosures
The authors have no financial conflict of interest.

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