Mast Cells Exert Effects Outside the Central Nervous System to Influence Experimental Allergic Encephalomyelitis Disease Course[1]

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Previous studies using mast cell-deficient mice (W/W^v) revealed that mast cells influence disease onset and severity of experimental allergic/autoimmune encephalomyelitis (EAE), the murine model for multiple sclerosis. The mast cell populations of these mice can be restored by transferring bone marrow-derived mast cells (BMMCs). Studies using the W/W^v reconstitution model have lead to major advances in our understanding of mast cell roles in vivo. However, despite its common use, details regarding the sites and kinetics of mast cell repopulation have remained largely uncharacterized. In this study, we examined the kinetics and tissue distribution of green fluorescent protein$^+$ BMMCs in reconstituted W/W^v mice to identify sites of mast cell influence in EAE. Reconstitution of naive animals with BMMCs does not restore mast cell populations to all organs, notably the brain, spinal cord, lymph nodes, and heart. Despite the absence of mast cells in the CNS, reconstituted mice exhibit an EAE disease course equivalent to that induced in wild-type mice. Mast cells are found adjacent to T cell-rich areas of the spleen and can migrate to the draining lymph node after disease induction. These data reveal that mast cells can act outside the CNS to influence EAE, perhaps by affecting the function of autoreactive lymphocytes. *The Journal of Immunology,* **2003, 171: 4385–4391.**

Mast cells are granulocytes conventionally recognized for their role in allergic inflammation. Although they reside predominately in tissues exposed to the external environment, including the skin, intestinal tract, and trachea, mast cells are also normally present in the heart, lymph nodes, spleen, and CNS (1). Mast cells can be activated by a multitude of stimuli, such as Abs, cytokines, chemokines, and neuropeptides, resulting in a variety of responses, including cell migration, the immediate release of inflammatory mediators, and selective cytokine production (2–7). Because mast cells are multifunctional cells with a widespread distribution, they are poised to play a pivotal role in the immune system.

The WBB6F$_1$-W/W^v mouse, commonly referred to as W/W^v, provides a useful tool for studying mast cell functions in vivo. These mice lack mast cells and exhibit severe macrocytic anemia as well as deficiencies in germ cell and melanocyte production. Mast cell precursors require the expression of c-*kit*, a transmembrane receptor with intrinsic tyrosine kinase activity, for their normal response to stem cell factor (SCF),[4] a major migration, proliferation, maturation, and survival factor (reviewed in Ref. 8). SCF is expressed in a variety of tissue microenvironments, including the bone marrow where mast cells normally begin their development. W/W^v mice result from a cross between strains of mice carrying two distinct naturally occurring mutations at the *c-kit* locus (9). The parental strains WB/Re-kit$^{W/+}$ and C57BL/kit$^{Wv/+}$ produce progeny of which one quarter are virtually mast cell-deficient (W/W^v) and have a mixed MHC haplotype, H-2^{bxj} (10). One half of the offspring are wild type (+/+) with both *c-kit* loci intact and can be used as mast cell-competent controls.

Because W/W^v mice have a defect in the cell surface expression of c-*kit*, it is possible to reconstitute these genetically defective mice with wild-type bone marrow to restore a mature mast cell population. Kitamura et al. (10) first demonstrated that at 105 days after bone marrow transplantation, mast cell numbers in the skin were half that of wild-type tissues, whereas various gastrointestinal tissues contained normal or increased numbers of mast cells compared with wild-type tissue. Importantly, the phenotypic characteristics of these cells resemble the native populations of mast cells in normal mice. However, bone marrow transplantation corrects all hematological disorders in these mice, including the macrocytic anemia. To assess the contribution of mast cells in isolation, a pure mast cell population must be transferred. Toward this end, wild-type bone marrow cells can be cultured in the presence of IL-3 to generate committed mast cell precursors (termed bone marrow-derived mast cells (BMMCs)). These cells can be injected i.v. or introduced to local sites, such as the gastrointestinal tract or skin, and differentiate faithfully in vivo (11, 12). Reconstitution with BMMCs leads to a selective and local correction of some of the mast cell defects, allowing a direct assessment of the contribution of mast cells to a given phenotypic outcome. These "mast cell knock-in" mice have been used in studies to unequivocally confirm the role of mast cells in several protective and pathologic responses. These include resistance to respiratory and intestinal bacterial infections, angiogenesis and effects on cardiac function, inflammation associated with the immediate and late phase of murine IgE-mediated cutaneous responses, and autoimmune diseases (8, 13–21).

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[4] Abbreviations used in this paper: SCF, stem cell factor; BMMC, bone marrow-derived mast cell; EAE, experimental allergic/autoimmune encephalomyelitis; GFP, green fluorescent protein; MOG, myelin oligodendrocyte gp; MS, multiple sclerosis.

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Despite its widespread usage, comprehensive studies of the reconstitution process have not been previously described. The time required for the establishment of mast cell populations in individual tissues as well as the changes in distribution that occur as reconstitution and disease progress are important factors to consider when studying the in vivo role of mast cells in the context of any disease model. Our laboratory recently used the W/W^v mouse model to demonstrate a critical role for mast cells in the pathogenesis of myelin oligodendrocyte gp (MOG)-induced experimental allergic/autoimmune encephalomyelitis (EAE). EAE is a rodent model of the human disease multiple sclerosis (MS) that shares many of its features. Both are debilitating diseases of the CNS characterized by perivascular inflammation, localized myelin destruction, and accumulation of immune cells within the CNS, culminating in impaired motor function (for review, see Ref. 22). Although MS and EAE result largely from CD4$^+$ T cell-driven autoimmune responses against self-myelin Ags, many other cell types contribute to disease pathogenesis. Following immunization with MOG_{33-55} peptide, W/W^v mice exhibit a delayed disease onset and a diminished disease severity compared with their wild-type littermates (23). The attenuated disease can be restored to wild-type levels following mast cell reconstitution. However, where and when mast cells exert their effects is unknown.

To investigate potential sites of mast cell influence during EAE, an analysis of kinetics and sites of mast cell reconstitution before and during disease was performed using green fluorescent protein (GFP$^+$) BMMC. These cells can be unequivocally identified by fluorescence microscopy, and thus provide a uniquely sensitive tool for cellular tracking. We demonstrate that reconstitution of naive animals with BMMCs does not restore mast cell populations to all organs, notably the brain, spinal cord, lymph nodes, and heart. Despite the absence of mast cells in the CNS, W/W^v mice reconstituted with GFP$^+$ BMMCs exhibit an EAE disease course that is equivalent to that induced in wild-type mice after immunization. Mast cells are found adjacent to T cell-rich areas of the spleen and can migrate to the draining lymph node after disease induction. These data reveal a role for mast cells acting outside the CNS to influence disease in this model of EAE. In addition, they demonstrate that mast cells are dynamic cells that can move to sites of initial T cell activation where they are likely to profoundly influence the generation of an adaptive immune response.

Materials and Methods

Mice

Female WBB6F$_1$-W/W^v mice and their wild-type littermates were purchased from The Jackson Laboratory (Bar Harbor, ME). GFP-transgenic C57BL/6 mice (kind gift of D. Archer, Emory University, Atlanta, GA) express GFP under the control of the actin promoter and have been previously described (24).

Bone marrow mast cell differentiation

Bone marrow cells were harvested from the femur and tibia in serum-free RPMI 1640 and differentiated in vitro as follows: briefly, cells were cultured for at least 6 wk in RPMI 1640 supplemented with 15% FBS, 0.5% penicillin/streptomycin, 1% L-glutamine, 1% sodium pyruvate, 20 μM 2-me, 30% WEHI-3B cell supernatant, and SCF (12.5 ng/ml), and were >95% c-*kit*^{high} and FcϵRI$^+$ before transfer.

Cell transfer into W/W^v mice

Six- to 8-wk-old W/W^v mice were injected via the tail vein with 5×10^6 BMMC or 1.8×10^7 whole bone marrow cells in PBS.

Analysis of mast cells in reconstituted organs

Organs were harvested and fixed in 4% paraformaldehyde for 6 h, then flash frozen using 2-methylbutane and OCT (Sakura Finetek, Torrance, CA). Tissue sections (12 μm) were cut using a cryostat. Tissues were analyzed for the presence of GFP using fluorescence microscopy. Alternatively, slides were stained with toluidine blue and then examined for presence of mast cell granules using a transmitted light microscope.

EAE disease induction and evaluation

Disease was induced with 300 μg MOG_{35-55} peptide (Microchemical Facility, Emory University) as previously described (23), with the exception of the day 7 injection of MOG, which was emulsified in IFA rather than CFA. The mice were scored daily for clinical signs of paralysis as follows: 0, no physical sign of disease; 1, tail flaccidity; 2, hind limb weakness; 3, hind limb paralysis; 4, fore limb paralysis and/or the inability to right from supine. For statistical analysis, repeated measures by ANOVA followed by Dunnett's posttests were performed.

Quantification of mast cell concentrations

The total number of mast cells was counted in four randomly chosen sections per organ. The area of each section was determined using the public domain NIH Image program (available at: http://rsb.info.nih.gov/nih-image/).

Results

The transfer of in vitro-differentiated BMMCs into W/W^v mice restores mast cell populations to only a subset of tissues

Bone marrow cells from a C57BL/6 mouse containing a GFP transgene under the transcriptional control of the actin promoter (24) were differentiated for 6–8 wk in vitro with IL-3 and SCF. The GFP$^+$ BMMCs are FcϵRI$^+$c-*kit*^{high}, and exhibit normal granular staining with metachromatic dyes such as toluidine blue (data not shown). A total of 5×10^6 GFP$^+$ BMMC were transferred into W/W^v mice via tail vein injection, and GFP$^+$ mast cell distribution was monitored in tissues from two mice each week using fluorescence (GFP$^+$) or transmission light microscopy (toluidine blue$^+$). The protocol is outlined in Fig. 1, and results are summarized in Table I.

GFP$^+$ mast cells are first detectable at 3 wk after transfer in the bone marrow, liver, lung, and spleen (Fig. 2, *a*, *b*, *c*, and *e*). In the spleen, mast cells are localized to the red pulp, and their frequency increases as the reconstitution progresses through wk 7, from 2.96 ± 1.35 cells/mm^2 at wk 3 to 13.78 ± 7.03 cells/mm^2 by wk 7. By wk 8 after reconstitution, the concentration of mast cells in the spleen is ~600-fold greater than that of a wild-type littermate (Fig. 2, *d* and *e*) (W/W^v: 178.0 ± 34.5 mast cells/mm^2, vs wild type: 0.3 ± 0.2 mast cells/mm^2). The splenic mast cell population then begins to contract (9.55 ± 5.06 mast cells/mm^2) at wk 16.

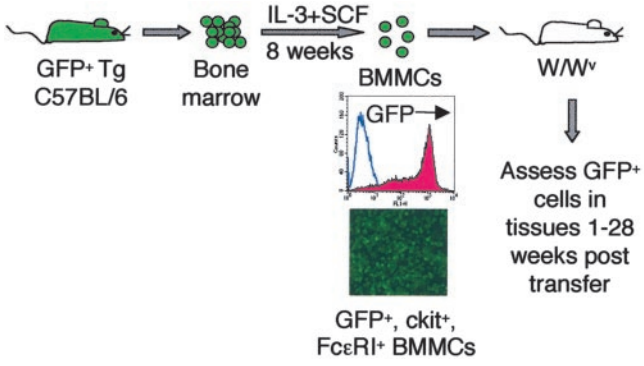

FIGURE 1. Schematic representation of the protocol for reconstitution of W/W^v mice with GFP$^+$ BMMCs. Bone marrow cells were harvested from C57BL/6 mice containing a GFP transgene expressed under the control of the actin promoter. Cells were cultured for 6–8 wk with IL-3 and SCF. The mast cell phenotype was confirmed by toluidine blue staining and analysis of cell surface c-*kit* and FcϵRI expression by flow cytometry. Cells were transferred to W/W^v mice by i.v. injection, and animals were assessed at various time points for GFP$^+$ mast cells in tissues.

Table I. *Summary of BMMC reconstitution kinetics*[a]

	Wk 1	Wk 3	Wk 5	Wk 7	Wk 8	Wk 9	Wk 10	Wk 16	Wk 28
Brain	ND[b]	ND	ND	No[b]	No	No	No	No	No
Bone marrow	No	Yes[c]	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Gut	No	No	No	Yes	Yes	Yes	Yes	No	No
Heart	No	No	No	No	No	No	No	No	No
Liver	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Lung	No	Yes	No	Yes	Yes	Yes	Yes	No	No
Lymph node	No	No	No	No	No	No	No	No	No
Skin	No	No	No	No	No	No	No	No	Yes
Spleen	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Spinal cord	ND	ND	ND	ND	ND	ND	ND	No	No

[a] For each time point, $n = 2$, except for weeks 16 and 28, $n = 1$.
[b] No, lack of detection of mast cells in each tissue.
[c] Yes, detection of mast cells in each tissue.

However, even at 28 wk after reconstitution, there are ~30-fold more splenic mast cells relative to wild-type animals that maintain relatively constant numbers of mast cells over time. Notably, at 16 and 28 wk, the mast cells appear in a clustered distribution in the spleen (Fig. 2*f*), perhaps reflecting clonal expansion of the population, as previously observed (25).

Other tissues require more time to acquire a detectable mast cell population. Mast cells were first observed in the small intestine at wk 7 (Fig. 2, *g* and *h*), whereas mast cells were not detected in the skin until wk 28 (Fig. 2*i*). In some organs mast cell reconstitution is only transient; reconstituted cells are no longer detectable in the small intestines or lung by wk 16. In addition, several organs that are normally populated by mast cells in wild-type animals are not reconstituted in this system. These include the brain, heart, inguinal lymph nodes, and spinal cord.

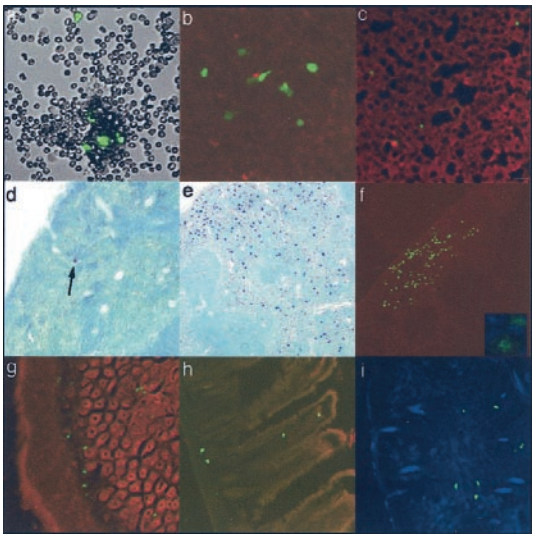

FIGURE 2. Representative sampling of tissues from W/W^v mice reconstituted with GFP$^+$ BMMCs. *a*, Overlay of FITC and phase-contrast images depicts GFP$^+$ BMMC in the bone marrow suspension 7 wk after injection. *b*, GFP$^+$ mast cells in the reconstituted liver. *c*, GFP$^+$ mast cells in reconstituted lung parenchyma. *d*, Mast cells, detected by toluidine blue staining, are present at a low frequency in the wild-type spleen. *e*, Mast cells (stained with toluidine blue) are numerous and widespread in the red pulp of the spleen at 8 wk after injection. *f*, By 16 wk after injection, mast cells in the spleen become highly clustered. *Inset*, Hoescht's stain was used to detect nuclei. Overlay of UV and FITC images confirms that the GFP$^+$ BMMCs are viable after reconstitution. *g*, GFP$^+$ mast cells in the duodenum. *h*, The small intestines at 10 wk after injection. *i*, GFP$^+$ mast cells in the skin at wk 28 after injection. Scale bar: *a* and *b* = 40 μm, *c–f* = 100 μm, *f* (*inset*) = 13 μm, *g–i* = 80 μm. GFP was detected using an FITC filter. The same panels were imaged using phase contrast (*a*), UV filter (*i*), or rhodamine filter (*b*, *c*, *f*, *g*, and *h*). The overlay of the FITC and rhodamine images confirms that the GFP$^+$ BMMCs are not artifacts of autofluorescence.

Whole bone marrow transfer into W/W^v mice restores most normal mast cell populations

To investigate the effects of haplotype variance (H-2^b donor cells into a H-2^{bxj} recipient) and BMMC differentiation on the ability of mast cell precursors to migrate and establish stable populations in vivo, we determined whether whole bone marrow derived from H-2^b mice could restore normal mast cell distribution. It was previously reported that the transfer of whole bone marrow from H-2^{bxj} wild-type littermates resulted in the repopulation of the skin, intestinal tract, and mesentery (10). Because mast cells arising from GFP$^+$ bone marrow would be indistinguishable from every other GFP$^+$ cell transferred, syngeneic C57BL/6 bone marrow was used, and mast cells were identified by toluidine blue staining. As shown in Table II, mast cell distribution in whole bone marrow-reconstituted mice more closely matches wild-type distribution than does a BMMC reconstitution. By wk 12 following bone marrow transfer, mast cells are restored to the spinal cord and inguinal lymph nodes, whereas the BMMC reconstitution does not repopulate these sites within the same time period (Fig. 3). Mast cells were not detected in the brain in these experiments, which is consistent with previous data by Johnson et al. (26) showing that 7 mo are required to reconstitute the brain following whole bone

Table II. *Transferring whole bone marrow into W/W^v mice yields a mast cell distribution more similar to a wild-type animal than does a BMMC reconstitution*[a]

	Wild Type	Whole BM wk 10	Whole BM wk 12	BMMC wk 10
Brain	Yes	No	No	No
Bone marrow	No	Yes	Yes	Yes
Gut	Yes	Yes	Yes	Yes
Heart	Yes	No	Yes	No
Liver	No	No	No	Yes
Lung tissue	No	No	No	Yes
Lymph node	Yes	Yes	Yes	No
Skin	Yes	Yes	Yes	No
Spleen	Yes	Yes	Yes	Yes
Trachea	Yes	Yes	Yes	No
Spinal cord	Yes	No	Yes	No

[a] For each whole bone marrow time point, $n = 1$.

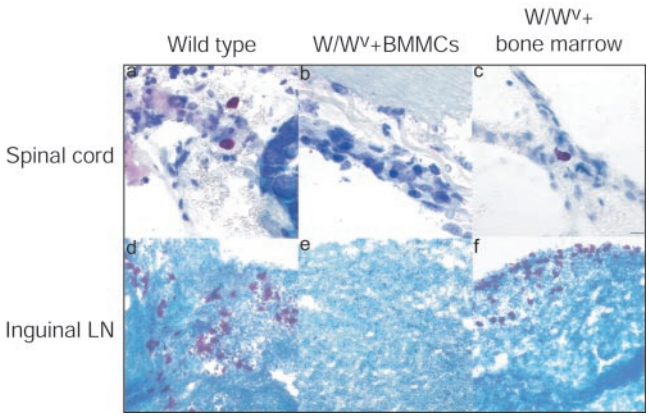

FIGURE 3. Comparison of mast cell distribution in the CNS and inguinal lymph node in wild-type, BMMC-reconstituted, and bone marrow-reconstituted W/W^v mice. Shown by toluidine blue staining, mast cells are present in wild-type mice in the meninges around the spinal cord (*a*) and in the inguinal lymph node (*d*). *b* and *e*, BMMC reconstitution does not restore mast cells to the CNS (spinal cord meninges shown here) or lymph node of W/W^v mice. *c* and *f*, Bone marrow transfer restores mast cells to the W/W^v spinal cord meninges and inguinal lymph nodes after 12 wk. Scale bar: *a–c* = 20 μm, *d–f* = 40 μm.

marrow transfer. Within some organs, the precise localization of mast cells is also more typical of that observed in a wild-type animal. For example, reconstitution with whole bone marrow mimics the natural distribution of mast cells in the murine respiratory tract, which is restricted to the trachea (27). In contrast, BMMC transfer results in the population of the lung parenchyma. In addition, reconstitution with whole bone marrow does not result in the increased number of mast cells in the liver or spleen observed in BMMC reconstitutions. These data confirm that rejection of H-2^b donor cells by the H-2^{bxj} W/W^v recipients does not occur. Instead, it is likely that the in vitro differentiation conditions used to generate BMMCs alter the migration potential of some mast cell precursors.

Establishment of stable mast cell populations following BMMC transfer into W/W^v mice is sensitive to pertussis toxin

Pertussis toxin is typically administered i.v. on days 0 and 2 during the EAE induction protocol, and is presumed to have a role in opening the blood-brain barrier, thus allowing inflammatory and autoreactive T cells to enter the CNS (28, 29). Thus, pertussis toxin may facilitate the entry of mast cells or their precursors into the CNS during disease induction. To examine this possibility and investigate the effects on mast cell migration to other sites, pertussis toxin (250 ng: standard dose used for EAE induction) was administered 1 day before injecting BMMCs. At wk 10 and 12 following BMMC transfer, analysis of tissue mast cells was performed using fluorescence microscopy and toluidine blue staining. No mast cells were detected in any tissue sites, including the CNS (data not shown). The inability to find mast cells using conventional metachromatic stains like toluidine blue confirms that pertussis toxin does not simply mask detection of the mast cells by inhibiting GFP production, but that it actually prevents mast cell reconstitution.

CNS mast cells do not contribute to EAE in reconstituted W/W^v mice

Previous experiments revealed that EAE disease course in wild-type and mast cell reconstituted W/W^v mice is virtually identical (23). Because mast cells are present in the CNS of wild-type mice, as well as in and around MS lesions (30–32), it was originally assumed that CNS mast cells have a direct and profound effect on the inflammatory events occurring in the CNS during these diseases. Although in our original study we detected an occasional CNS mast cell in reconstituted W/W^v mice during the late phase of EAE, the transfer of GFP$^+$ BMMCs did not result in detectable CNS mast cells at any time point analyzed. We speculate that the repopulation of mast cells in the CNS following BMMC transfer is a very rare occurrence. Alternatively, a phenotype difference may exist between GFP$^+$ (H-2^b)-derived mast cells and those from wild-type littermates of the W/W^v (H-2^{bxj}) mice that accounts for the complete exclusion of GFP$^+$ cells from the CNS. To test the capacity of GFP$^+$ mast cells to function in vivo, GFP$^+$ or wild-type BMMCs were transferred into W/W^v mice. Eight weeks after reconstitution, EAE was induced in the reconstituted mice, as well as in age-matched female W/W^v and their wild-type littermate controls, and the mice were scored daily for clinical signs of disease. Consistent with our previous findings, the W/W^v mice exhibited a delayed disease onset and less severe disease course than their mast cell-competent wild-type littermates. GFP$^+$ BMMC reconstitution restored disease to wild-type levels. The mean daily clinical score of the GFP$^+$ BMMC-reconstituted mice was statistically equivalent ($p = 0.87$) to both the wild-type and the wild-type-BMMC-reconstituted mice (Fig. 4). The ability of GFP$^+$ BMMCs to restore a wild-type EAE phenotype demonstrates that no functional defects are inherent in these cells.

Mast cell numbers in the secondary lymphoid organs change as EAE disease progresses

The previous observations suggest that mast cells act in the periphery to influence disease course. However, it is possible that mast cells may gain entry into the CNS of reconstituted mice only after disease induction. To investigate potential changes in mast cell distribution during the course of acute EAE, disease was induced in GFP$^+$ BMMC-reconstituted W/W^v mice. CNS and lymphoid tissues were harvested and analyzed for the presence of mast cells at different stages of disease severity. Mast cells were not detected in the brain or spinal cord at any point during the course of disease ($n = 6$), further supporting a mechanism of action outside the CNS. Given the importance of the CNS in this disease

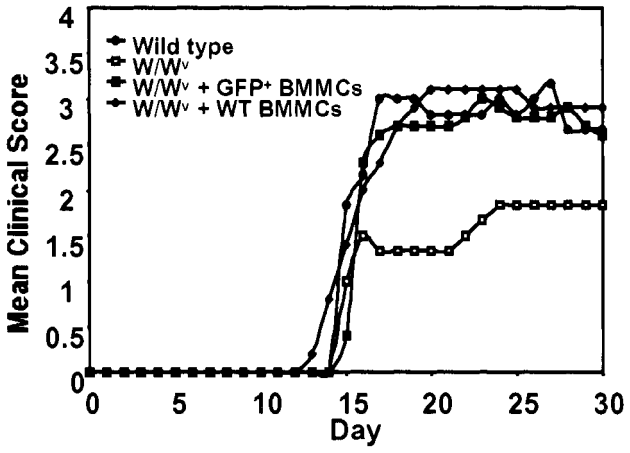

FIGURE 4. GFP$^+$ BMMC (H-2^b)-reconstituted W/W^v mice exhibit a similar disease course compared with those reconstituted with wild-type (WT) BMMCs (H-2^{bxj}) and wild-type littermates ($n = 5$). Mast cell-specific reconstitution was confirmed by hematocrit, indicating that the W/W^v macrocytic anemia was not corrected (W/Wv: 39.6 ± 2.2; W/W^v + WT BMMCs: 42.0 ± 1.7; W/W^v + GFP$^+$ BMMCs: 38.1 ± 2.8; Wild type: 57.7 ± 3.0) as previously described (23).

model, we used additional techniques to conclusively determine that mast cells were not present in the CNS. In the brain, as the meninges were a likely potential site for reconstitution, we verified that tissue sections contained an intact meningeal layer visible by toluidine blue or Hoescht's staining. To examine the meninges independently, we perfused two EAE-induced reconstituted W/W^v mice, isolated the meninges, and examined the layer as a whole-mount. No mast cells were detected in the CNS using this technique. In the spinal cord, the meninges tended to loosen during sample preparation, which enhanced our ability to detect meningeal mast cells in that organ. However, we did not detect mast cells in the meninges of any BMMC-reconstituted animal.

We did observe a striking decrease in mast cell frequency in the spleen as disease severity increased. Mast cell concentrations were quantified for one animal at each stage of acute disease. In a naive animal 8 wk after reconstitution, 178.0 ± 34.5 mast cells/mm^2 were detected (Fig. 5*a*). At day 11 after disease induction, before the onset of clinical disease, degranulated mast cells were observed in the spleen (Fig. 5*d*), indicative of mast cell activation. At day 17 following disease induction (clinical score = 1), mast cells were still numerous in the spleen (39.08 ± 1.91 mast cells/mm^2) with some located at the interface of the red pulp and the white pulp (data not shown). The number of splenic mast cells decreased with increasing disease severity, with 3.6 ± 1.9 mast cells/mm^2 at a disease score of 2.5 (Fig. 5*b*) and 0.5 ± 0.3 mast cells/mm^2 at a disease score of 3 (Fig. 5*c*). The animal with the most severe disease (clinical score = 4) had very few detectable mast cells in the spleen (0.3 ± 0.1 mast cells/mm^2, data not shown). This phenomenon was not a result of normal mast cell clearance, because naive reconstituted animals maintained elevated mast cell levels throughout the same time period. A decrease in splenic mast cell frequency between naive and diseased wild-type littermates was also observed (data not shown and Ref. 30). Notably, although mast cells are not detected in the lymph node of naive reconstituted animals (Fig. 5*e*), numerous mast cells were detected in the draining inguinal lymph node of an animal with severe disease (clinical score = 4, Fig. 5*f*).

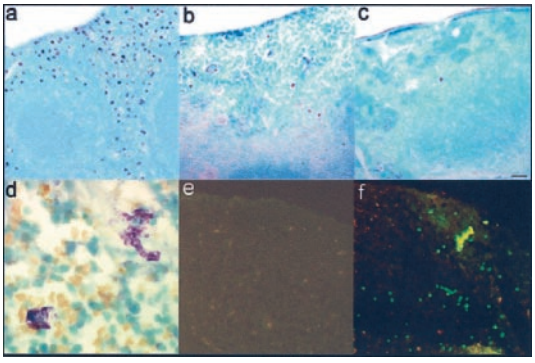

FIGURE 5. Changes in mast cells in secondary lymphoid organs during EAE disease progression. *a*, In the spleen of an unimmunized reconstituted W/W^v animal at wk 8 after injection, mast cell frequency was 178.0 ± 34.5 mast cells/mm^2. *b*, At day 19 after immunization, at a clinical disease score of 2.5, mast cell frequency was 3.6 ± 1.9 mast cells/mm^2. *c*, At day 34 (score = 3), mast cell frequency was 0.5 ± 0.3 mast cells/mm^2. *d*, Degranulated mast cells present in the reconstituted spleen at day 11, indicating prior cell activation. *e*, BMMC-reconstituted inguinal lymph node at day 17 (score = 2.5) following disease induction does not contain mast cells. *f*, In a BMMC-reconstituted animal with severe disease (score = 4), mast cells are present in the inguinal lymph node. Scale bar: *a–c* = 100 μm, *d* = 10 μm, *e* and *f* = 80 μm.

Discussion

It is becoming increasingly evident that the contribution of mast cells to both physiological and pathologic processes extends far beyond their accepted role in allergic disease (19, 33, 34). The W/W^v reconstitution mouse model is widely used for assessing these additional roles. Differences between reconstituted and wild-type mice must be taken into account when interpreting data from such studies, as the distribution and number of cells present in an organ may alter the quality and magnitude of an immune response. In this study, we examined the distribution of mast cells at various times after BMMC transfer in both naive and immunized W/W^v mice. We demonstrate that the i.v. transfer of bone marrow mast cells generated by in vitro differentiation with SCF and IL-3, a commonly used protocol, does not result in the faithful repopulation of all tissue sites. These findings highlight differences in the stability and frequency of mast cell populations that occur with time. Although extremely valuable in some experimental settings, use of the W/W^v mouse model to study in vivo contributions of mast cells is limited by the selective reconstitution and the transient nature of some mast cell populations. Studies to assess mast cell involvement in CNS and cardiac function, for example, will require the identification of factors that give rise to cells capable of stably populating these tissue sites.

Two observations indicate that BMMC reconstitution is an active process and not merely the result of the mast cells becoming lodged in these tissues following their initial injection into the circulation. First, mast cell populations such as those in the spleen and liver are maintained for extensive times after transfer in naive animals. Second, treatment of mice with pertussis toxin before mast cell transfer prevents reconstitution of these organs. Because pertussis toxin inhibits G protein-coupled signaling, the data indicate that these pathways are necessary for the establishment of mast cells in tissue sites. Data from previous studies demonstrating the dependence of mast cell migration on G protein-coupled signaling in response to chemotactic stimuli (35) are consistent with this idea.

The observation that transfer of whole bone marrow, but not differentiated mast cell precursor cells, can restore a relatively normal mast cell phenotype to W/W^v mice indicates that the in vitro culture conditions direct differentiation of select subpopulations of mast cell precursors with a limited ability to establish stable populations in some tissues. This may be caused by the lack of expression of necessary homing receptors that direct tissue-specific migration. BMMCs lacking $\alpha_4\beta_7$ integrin, for example, cannot home to the small intestine but do repopulate the large intestine and other organs (36). Alternatively, cells may migrate to appropriate target tissues, but may be unresponsive to the local differentiation factors required for permanent establishment of these cell populations.

Despite the absence of mast cells in the CNS of reconstituted W/W^v mice, these animals exhibit an EAE disease course that is statistically equivalent to that of their wild-type littermates. For many years it has been appreciated that mast cells are normally present in the brain and spinal cord and can be found along the periphery of MS and EAE lesions (30–32). The apparent correlation between the colocalization of mast cells with demyelinated lesions is consistent with the idea that mast cells impact EAE disease course, perhaps through the release of TNF-α, histamine, and proteases directly within the CNS. Support for this idea comes from a recent study showing that expression of transcripts encoding mast cell-specific genes, including tryptase III, histamine receptor 1, and FcR, are significantly increased in the plaques of patients with chronic MS (37). Our findings do not rule out the

possibility that mast cells directly influence inflammatory events in the CNS of wild-type mice or in mast cell-competent individuals. However, they do elucidate a role for mast cell influence in the periphery.

We demonstrate that mast cells are present in the secondary lymphoid organs of both wild-type and reconstituted W/W^v mice and undergo disease-induced changes in numbers and distribution. The apparent decrease in mast cells in the spleen as EAE progresses is not the result of a general loss of mast cells caused by normal phagocytosis or clearance, because naive reconstituted mice maintain their mast cell populations within this organ for at least 28 wk. The reduction in splenic mast cell numbers may reflect apoptosis following activation in the spleen as a means of controlling the inflammatory response analogous to events that occur in the intestines during *Trichinella spiralis* infection (38). Alternatively, consistent with previous evidence that mast cells migrate to secondary lymphoid organs in response to immune challenge (6, 7), mast cells may undergo directed migration into peripheral tissues such as the lymph nodes. Although not detected in a naive reconstituted W/W^v mouse, numerous mast cells were also present in the draining inguinal lymph node of an animal with severe clinical disease. Because mast cells are present at sites where initial T and B cell responses are generated, it is likely that they can directly influence these events. Some recent results from T cell transfer experiments support the idea that the mast cell "microenvironment" can influence the generation of MOG-specific T cells during early activation events, and also demonstrates a role for mast cells after initial T cell priming.[5] First, adoptive transfer of W/W^v-derived-encephalitogenic T cells (those primed in a mast cell-deficient environment) elicit less severe EAE than do encephalitogenic T cells generated in wild-type mice. Secondly, EAE disease course was compared in wild-type and mast cell-deficient recipients after adoptive transfer of wild-type encephalitogenic T cells. Despite the transfer of equivalent numbers of MOG-specific T cell blasts, W/W^v recipients consistently exhibit less severe disease. To demonstrate that these differences are not caused by intrinsic defects in W/W^v T cells, naive T cells from W/W^v mice or their wild-type littermates were transferred to TCR$\beta^{-/-}$ recipients. After MOG-immunization, both groups exhibit virtually identical disease courses, indicating that W/W^v T cells are fully competent to mediate severe disease if generated in a mast cell-competent environment.

Although the initial trigger for the onset of MS is unknown, it has been proposed that infection or injury in genetically predisposed individuals may play a role in activating an autoreactive T cell response (for review, see Refs. 39 and 40). In a wild-type setting, the widespread distribution of mast cells at the interface between the tissue and the external environment allows these cells to be a first defense against invading pathogens. We hypothesize that stimulation of mast cells in the course of certain infections may contribute to a proinflammatory environment that leads to activation and proliferation of quiescent, autoreactive T cells. This may occur through direct mast cell interaction with bacterial or viral components via Toll-like or other receptors (41, 42). At the site of Ag entry, local release of mast cell cytokines may influence dendritic cell maturation, which in turn can alter T cell differentiation fates. Within the secondary lymphoid organs, mast cells may provide an early burst of IL-4, IFN-γ, or histamine, all of which have been shown to directly influence the generation of proinflammatory T cells and B cells (43–45). Studies showing that mast cells are capable of Ag presentation (46, 47) raise the possibility that mast cells can serve as APCs to activate myelin-specific T cells. In addition, mast cells release chemokines such as macrophage-inflammatory protein-1β, a potent T cell chemoattractant (6, 7). Collectively, these functions could influence the autoimmune response and ensuing tissue damage. Given their varied mechanisms of action, it is likely that mast cells function in the development of other adaptive immune responses, including antimicrobial and anti-tumor responses. Studies are underway to further define mast cell functions in vivo.

[5] M. Robbie-Ryan and M. A. Brown. Mast cells are required for optimal autoreactive T cell responses in EAE. *Submitted for publication.*

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