PERGAMON

Molecular Immunology 38 (2001) 1373–1378

Molecular Immunology

www.elsevier.com/locate/molimm

Review

Mechanisms underlying mast cell influence on EAE disease course

Melissa A. Brown [a,b,*], Melinda B. Tanzola [b], Michaela Robbie-Ryan [b]

[a] *Department of Pathology, Emory University School of Medicine, WMB 7311, 1639 Pierce Drive, Atlanta, GA 30322, USA*
[b] *Graduate Program in Immunology and Molecular Pathogenesis, Atlanta, GA 30322, USA*

Received 3 January 2002; accepted 28 February 2002

Abstract

It is well established that $CD4^+$ T cells are of central importance in mediating the autoimmune destruction associated with the neurological demyelinating disease Multiple sclerosis (MS) and the rodent model of MS, EAE (experimental allergic encephalomyelitis). However, other cells also play a critical role in the inflammatory events that lead to the varying degrees of myelin and axonal damage observed in this disease syndrome. In this review, we present evidence that mast cells, best studied in the context of allergic disease, contribute to EAE disease pathology. Using mast cell-deficient mice, we demonstrate that mast cells are necessary for the full manifestation of MOG-induced EAE disease and show that cross-linking of Fc receptors is one mechanism of mast cell activation in disease. In addition, we provide evidence that mast cells exert influences outside the CNS, perhaps through the effects on the generation of the anti-MOG T cell response.

Keywords: EAE disease; Multiple sclerosis; Mast cells; CNS; Autoimmunity; Fc receptors; Mast cell-deficient mice

1. Introduction

Experimental allergic encephalomyelitis (EAE), the prototypical rodent model of human multiple sclerosis (MS), is a $CD4^+$ T cell-mediated autoimmune disease characterized by inflammation in the CNS (for review, see Steinman, 1996). It is associated with an early breach of the blood brain barrier, focal perivascular mononuclear cell infiltrates, gliosis, and in some models, demyelination of the CNS white matter. A major autoimmune response against myelin proteins and/or myelin-producing cells of the CNS (oligodendrocytes) leads to demyelination, a hallmark of these diseases. Adoptive transfer of encephalitogenic, $CD4^+$ T cells specific for myelin proteins can transfer disease to syngeneic, naive hosts in susceptible strains, such as SJL/J mice and Lewis rats (Bernard et al., 1992). It has been proposed that direct myelin destruction is carried out by activated immune cells, such as resident microglia, infiltrating macrophages and $CD8^+$ T cells under the influence of regulatory $CD4^+$ Th1 cells. Autoantibodies are also thought to contribute to the pathology associated with EAE, however adoptive transfer of autoantibodies alone does not transfer disease (Cross et al., 2001). In the following discussion, we review our studies using a mast cell-deficient mouse model that demonstrate mast cells also make a significant contribution to the pathology associated with EAE and provide information on the myriad of ways they may exert their influence.

2. Mast cell overview

Mast cells are derived from $CD34^+$ hematopoietic pluripotent stem cells present in the bone marrow (Rodewald et al., 1996). Committed mast cell precursors bearing c-kit, the receptor for stem cell factor (SCF), and FcεRI, the high affinity IgE receptor, migrate to target tissues where they can develop into distinct phenotypes under the influence of the unique array of differentiation and growth factors present. Mast cells are prevalent in the skin, mucosa of the genitourinary, respiratory and gastrointestinal tracts as well as the CNS (see below) and are most often found adjacent to blood vessels and peripheral nerves. This widespread distribution, particularly at sites most vulnerable to entry of infectious organisms, suggests the potential of mast cells to act as a first line of defense in fighting infection (Galli and Wershil, 1996). A hallmark of mast cells is their ability to immediately release a number of preformed mediators

* Corresponding author. Fax: +1-404-727-5764.
E-mail address: mbrow18@emory.edu (M.A. Brown).

0161-5890/02/$ – see front matter
PII: S0161-5890(02)00091-3

upon activation. Included among these are histamine, serotonin, kinins and proteases. Together with a number of induced mediators, such as prostaglandins and leukotrienes, these mast cell-derived molecules can enhance vascular permeability, recruit inflammatory cells into areas of local immune reactivity and cause direct tissue damage. Mast cell activators include Ig–Ag complexes, neuropeptides, such as substance P (Ansel et al., 1993), as well as bacterial products, such as LPS and FimH (Malaviya et al., 1994; Nygen and Dahlen, 1981).

3. Mast cells are present in the CNS

Although not as numerous as some of the infiltrating cell types in the CNS during acute EAE and MS disease, extensive data demonstrates mast cells are present in the central nervous system. In the brain, mast cells are most prevalent in the leptomeninges, the thalamus, hypothalamus and spinal cord dura mater where they are closely associated with blood vessels and nerves (reviewed in Johnson and Krenger, 1992; Silver et al., 1996; Zhuang et al., 1997). They are also predominant as periganglionic rings around dorsal root ganglia (DRG) and within dorsal and ventral roots proximal to the DRGs.

4. Indirect evidence that mast cells are associated with MS/EAE

The idea that mast cells contribute to the pathology associated with MS is not a new one. There are numerous studies showing a correlation between mast cell number and/or distribution and MS or EAE. Over 100 years ago, an association of mast cells with CNS plaques in MS patients was noted (Neuman, 1890). This has been confirmed in several subsequent studies. In both the murine model of MS and the human disease, it was shown that sites of inflammatory demyelination are also sites of mast cell accumulation in the brain and spinal cord (Bebo et al., 1996; Dines and Powell, 1997; Ibrahim et al., 1996; Orr, 1988). In acute EAE, the percentage of degranulated mast cells increased with the clinical onset of disease symptoms (Brenner et al., 1994). Tryptase, a mast cell-specific proteolytic enzyme, is elevated in the cerebrospinal fluid of MS patients (Rozniecki et al., 1995). A functional connection between mast cells and CNS damage is inferred in studies that show isolated mast cell-derived proteases can degrade myelin (Dietsch and Hinrichs, 1991; Johnson et al., 1988a) and that myelin can directly stimulate mast cell degranulation (Brenner et al., 1994; Johnson et al., 1988b; Orr, 1988). Finally, there are reports that mast cell stabilizing drugs improve symptoms in both the human and rodent form of MS (Brosnan and Tansey, 1984; Dietsch and Hinrichs, 1989; Seeldrayers et al., 1989). However, all of these studies are only correlative and do not *definitively* implicate the mast cell in the pathology of MS/EAE.

5. Potential mechanisms of mast cell action

There are a number of ways that mast cells can influence disease course (summarized in Fig. 1). For example, the proximity of mast cells to blood vessels and nerves is likely to be significant. Through the release of vasoactive amines, such as histamine, mast cells can profoundly impact vascular permeability and open the blood brain barrier allowing greater influx of activated T cells. Moreover, mast cell proteases may directly damage the myelin sheath or the nerves. The cytokines and chemokines expressed by mast cells, including IL-4, IFN-γ, TNF-α and MIP1-α may affect immune cell trafficking through direct chemotaxis and/or influence adhesion molecule expression on the endothelium. The ability of mast cells to migrate to secondary lymphoid organs also raises the possibility that they can regulate the induction and/or amplification of a polarized Th response (Friend et al., 2000; Wang et al., 1998).

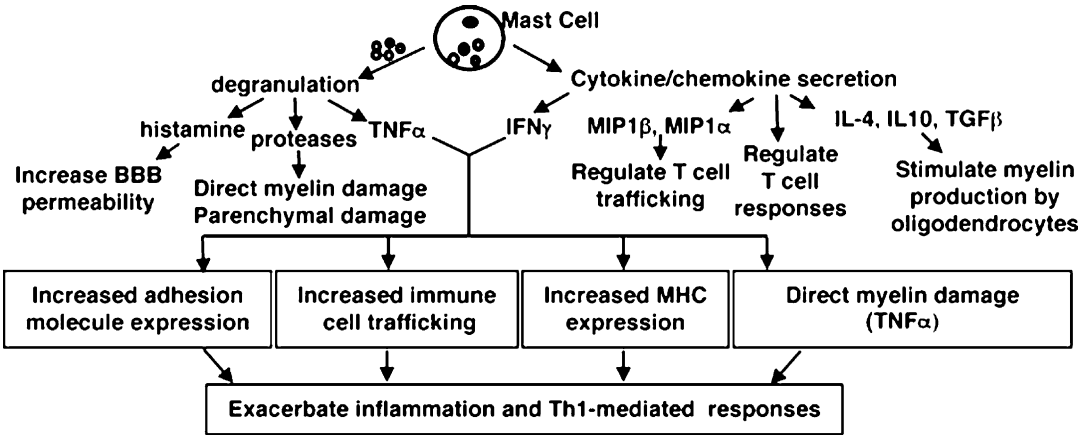

Fig. 1. Mast cells can influence the autoreactive inflammatory response associated with EAE in a variety of ways. These influences can occur both within and outside the CNS.

6. Direct evidence that mast cells contribute to EAE

The definitive demonstration that mast cells contribute to EAE was dependent on the use of mast cell-deficient (W/W^v: c-kit^W/c-kit$^{W^v}$) mice. W/W^v mice have two distinct mutations in c-kit, a transmembrane receptor with intrinsic tyrosine kinase activity required for the normal response to stem cell factor, a major migration, proliferation, maturation and survival factor (reviewed in Galli et al., 1992). This impairment in SCF signaling has the most profound effects on the development of mast cells. These mice also exhibit anemia, have deficits in skin melanocytes and are sterile.

It is possible to reconstitute genetically defective W/W^v animals with either wild type bone marrow or in vitro differentiated mast cell precursors. In vitro, the culture of wild type bone marrow cells with IL-3 (+/− SCF) generates a population of committed mast cell precursors (termed BMMC) which, when transferred to mast cell-deficient hosts, differentiate faithfully in vivo (Nakano et al., 1985). The cells can be injected intravenously or at local sites, such as the GI tract or lung. Reconstitution with these committed precursor cells leads to a *selective* and *local* correction of the mast cell defect allowing a direct assessment of the contribution of mast cells to a given phenotypic outcome. These so called "mast cell knock-in" mice have been used in several studies to unequivocally confirm the role of mast cells in several physiologic responses and disease states (reviewed in Galli et al., 1992).

We have used myelin oligodendrocyte glycoprotein (MOG) to induce disease. MOG, comprises only ∼0.05% of myelin proteins, but is responsible for the induction of a robust antibody response that mediates demyelination in the CNS (Bernard et al., 1997). MOG and $MOG_{35–55}$ (MEVGWYRSPFSRVVHLYRNGK), a peptide derived from this protein, can induce typical EAE disease in C57BL/6J mice and other H-2^b strains (Mendel et al., 1995). Because many of the targeted disruptions of immune response-related genes are on the H-2^b background, this significant finding opened new avenues of investigation in EAE research Importantly for our studies, W/W^v mice (H-2^{bxj}) are semi-syngeneic with B6. Thus, this system has provided our laboratory with a useful model to study the effects of mast cells on EAE disease course.

7. Mast cell-deficient mice exhibit delayed onset and decreased EAE disease severity when compared to wild type littermates

EAE was induced in mast cell-deficient mice and their wild type littermates (Secor et al., 2000). As shown in Fig. 2, the mast cell-deficient animals develop much less severe disease and delayed onset compared to wild type littermates. The disease incidence was significantly lower as well. Only 7 out of 17 W/W^v animals showed clinical symptoms, whereas 15 out of 16 wild type mice developed disease.

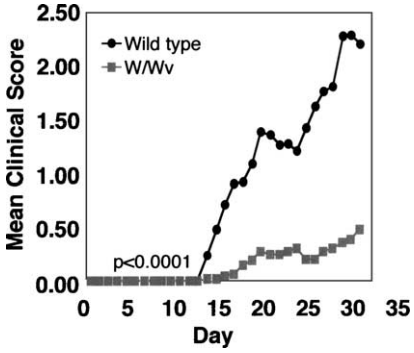

Fig. 2. W/W^v mice exhibit less severe disease that wild type animals. EAE was induced in mast cell-deficient mice and their wild type littermates as described (Mendel et al., 1995). Briefly, 300 μg of $MOG_{35–55}$ peptide per 100 μl PBS emulsified in an equal volume of complete Freund's adjuvant/0.5 mg/ml of Mycobacterium tuberculosis was injected subcutaneously in the flank on Days 0 and 7. Pertussis toxin (250 ng/500 μl) was administered intravenously per tail vein on Days 0 and 2. Clinical scores were assigned daily to each mouse. Scoring: 0—no clinical disease; 1—tail flaccidity; 2—hind limb weakness; 3—hind limb paralysis; 4—fore limb paralysis or loss of ability to right from supine; 5—death. Mean high score: W/W^v, 0.61 vs. WT, 2.42, $P < 0.008$; mean day of onset: W/W^v, 24.3 vs. WT, 18.0, $P < 0.03$ (as reported in Secor et al., 2000).

8. Reconstitution of mast cell-deficient mice with bone marrow derived mast cells restores susceptibility to EAE

To restore the mast cell population, in vitro differentiated c-kit$^+$ and FcεRI$^+$ BMMC were injected intravenously into W/W^v mice. At 10–12 weeks, reconstitution was confirmed in a subset of mice by histological analysis of several tissues including the lung, gut, bone marrow and spleen. EAE was induced and disease scored as described in Fig. 2. Mast cell-reconstituted mice exhibited disease scores comparable to wild type mice (Fig. 3). Transfer of BMMCs does not

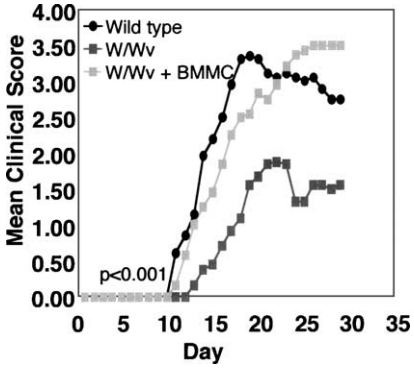

Fig. 3. Reconstitution of the mast cell population restores disease susceptibility to W/W^v mice. In vitro differentiated mast cells (>97% FcεRI$^+$, c-kit$^+$) were injected intravenously into mast cell-deficient mice. Ten weeks post BMMC transfer; disease was induced and scored as described in Fig. 2. Mean high score: W/W^v, 1.63 vs. WT, 3.45, W/W^v +BMMC, 3.75, $P < 0.002$; mean day of onset: W/W^v, 18.0 vs. WT, 12.4 and W/W^v +BMMC, 13.1, $P < 0.008$ (as reported in Secor et al., 2000).

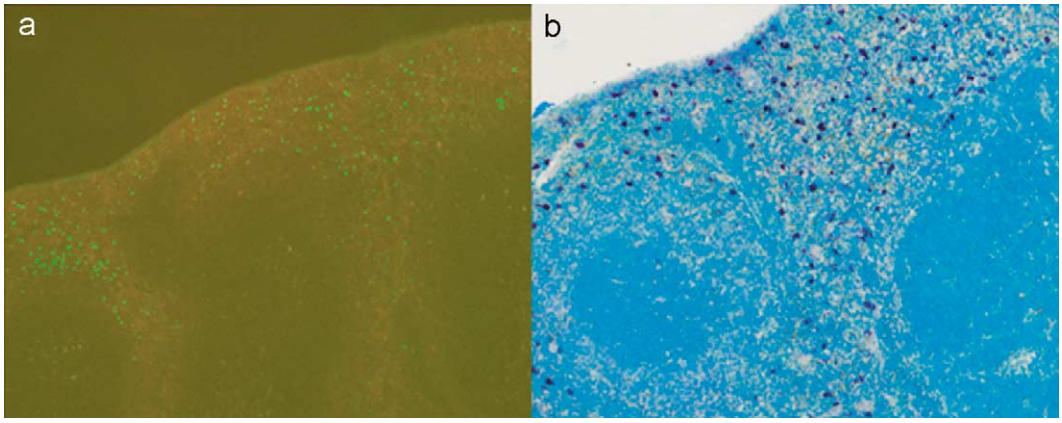

Fig. 4. Detection of GFP-expressing mast cells in the red pulp of the spleen. Panel (a): fluorescence microscopy showing GFP+ mast cells that reside in the spleen 8 weeks post-intravenous transfer. Panel (b): parallel section stained with toluidine blue.

correct the associated anemia confirming that the reconstitution is selective for the mast cell population.

Three questions are the focus of our current investigative efforts:

(1) What are the sites of mast cell action?

Most previous studies to verify sites of mast cell reconstitution have relied on the histological examination of tissues at 10–12 weeks post cell transfer. Because mast cells are relatively rare in tissues, these techniques are tedious and relatively insensitive. To overcome these obstacles, mast cell-deficient mice were reconstituted with bone marrow mast cells isolated from GFP transgenic mice. This transgene expresses GFP in all tissues under the control of an actin promoter. At weekly intervals, tissues were harvested and examined for GFP expression using fluorescence microscopy. This has proven to be a very sensitive method to track the efficiency of mast cell reconstitution. An example of this analysis is shown in Fig. 4. By 7 weeks post BMMC transfer, most tissues, including the gut, lung, and spleen were repopulated with mast cells. Of note, mast cells were *not* detected in the heart, skin or CNS at 10 weeks post transfer. This finding may reflect inefficient reconstitution to these sites due to the in vitro differentiation conditions used, conditions which fail to generate mast cell precursors with appropriate homing receptors. Currently, little is known about the signals that lead to the localization of mast cells to the CNS. Alternatively, the establishment of CNS mast cell populations may be delayed or require immune challenge. Studies to distinguish between these possibilities are underway. These results have important implications regarding the mechanism of mast cell action in this experimental model. The inability of BMMC to repopulate the CNS in this experimental setting, under conditions where they restore disease susceptibility indicates that mast cells are acting outside the CNS.

(2) How are mast cells activated in disease?

Anti-MOG antibodies of the IgG isotype are prevalent in multiple sclerosis as well as in the rodent form of disease (Cross et al., 2001). Thus, it is possible that mast cells are activated through specific Ig receptors. In addition to the high affinity IgE receptor (FcεRI), murine mast cells express the activating IgG receptor FcγRIII, as well as the inhibitory receptor FcRIIB. To examine the role of antibody-mediated mast cell activation in EAE, W/W^v mice were reconstituted with $Fc\gamma R^{-/-}$ BMMC which lack the common γ signaling chain and do not express activating Fc receptors. Ten weeks later, the animals were subject to disease induction. As shown in Fig. 5, mice reconstituted with $Fc\gamma R^{-/-}$ mast cells exhibit a disease course that parallels that of W/W^v mice. That is, these cells are unable to restore susceptibility to

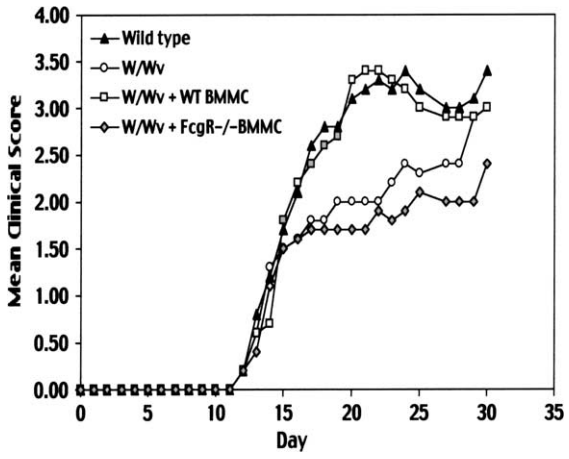

Fig. 5. Reconstitution with $Fc\gamma R^{-/-}$ BMMC does not restore disease susceptibility to W/W^v mice. $Fc\gamma R^{-/-}$ BMMC were transferred IV to W/W^v mice. Disease was induced 10 weeks post-transfer. Individual animals were scored for disease as described in Fig. 2. Mean high score: W/W^v, 2.8 and W/W^v, $+Fc\gamma R^{-/-}$ BMMC 2.9 vs. WT, 3.4 and W/W^v + wild type BMMC, 3.5.

severe disease. Although we cannot exclude the possibility that Ig-independent mechanisms of mast cell activation exist in this disease, these data support the idea that Ig-dependent mast cell activation is critical for mast cell effector function. Experiments to assess the specific contribution of the FcγRIII and FcRIIB receptors are underway.

(3) How do mast cells influence disease?

As discussed previously, mast cells are important sources of many cytokines and other mediators that have been implicated either directly or indirectly in MS and EAE. Their presence in the CNS of normal animals is consistent with the idea that they regulate recruitment of inflammatory cells and mediate direct myelin damage. However, the lack of appreciable numbers of CNS mast cells in our reconstitution model suggests another possibility. That is, mast cells may also influence the generation of the anti-MOG T cell response and, through chemokine expression, direct T cell migration to target sites. Mast cells reside in the spleen and when activated under certain conditions, can migrate to lymph nodes. This places them at sites where T cells first encounter antigen and undergo further differentiation and clonal expansion (Friend et al., 2000; Lin et al., 2000; Wang et al., 1998). In addition, mast cells express IL-4 and IFN-γ, two cytokines that govern the differentiation of $CD4^+$ T cells to Th1 and Th2 cells. We have recently initiated studies to explore these possibilities. To verify that there is no inherent defect in W/W^v T cells, wild type or W/W^v T cells were adoptively transferred into T cell-deficient mice ($TCR\beta^{-/-}$) prior to MOG immunization. In this setting, W/W^v T cells are able to induce disease equivalent to wild type cells (Robbie-Ryan, unpublished data). These data indicate that in a mast cell competent environment, W/W^v T cells can function normally to cause disease. Preliminary results from studies to compare sites of T cell migration and activation marker expression post MOG immunization indicate there are differences in T cell trafficking patterns and activation state in wild type and mast cell-deficient mice (Robbie-Ryan, unpublished data). These differences may contribute to a less robust autoreactive T cell response in W/W^v mice that results in less severe disease. Studies to fully characterize these differences and examine $CD4^+$ T cell polarization in these animals are currently in progress.

In summary, the data presented here add to the growing body of evidence that mast cells have profound effects on a wide variety of physiological processes. In addition to their well-defined role in allergic inflammation and as a first line of defense against some infectious agents, the possibility that mast cells affect adaptive immune responses implies that their influence in protective and pathologic immune responses is much greater than previously appreciated.

Acknowledgements

This work was supported by a grant from the National Multiple Sclerosis Society.

References

Ansel, J.C., Brown, J.R., Payan, D.G., Brown, M.A., 1993. Substance P selectively activates *TNF-α* gene expression in murine mast cells. J. Immunol. 150, 4478–4485.

Bebo Jr., B.F., Yong, T., Orr, E.L., Linthicum, D.S., 1996. Hypothesis: a possible role for mast cells and their inflammatory mediators in the pathogenesis of autoimmune encephalomyelitis. J. Neurosci. Res. 45, 340–348.

Bernard, C.C.A., Mandel, T., Mackay, I.R., 1992. Experimental models of human autoimmune disease: overview and prototypes. In: Rose, N.R., Mackay, I.R. (Eds.), The Autoimmune Diseases II. Academic Press, San Diego, pp. 47–106.

Bernard, C.C.A., Johns, T.G., Slavin, A., Ichikawa, M., Ewing, C., Liu, J., Bettadapura, J., 1997. Myelin oligodendrocyte glycoprotein: a novel candidate autoantigen in multiple sclerosis. J. Mol. Med. 75, 77–88.

Brenner, T., Soffer, D., Shalit, M., Levi-Schaffer, F., 1994. Mast cells in experimental allergic encephalomyelitis: characterization, distribution in the CNS and in vitro activation by myelin basic protein and neuropeptides. J. Neurol. Sci. 122, 210–213.

Brosnan, C.F., Tansey, F.A., 1984. Delayed onset of experimental allergic neuritis in rats treated with reserpine. J. Neuropath. Exp. Neurol. 43, 84–93.

Cross, A.H., Trotter, J.L., Lyons, J., 2001. B cells and antibodies in CNS demyelinating disease. J. Neuroimmunol. 112, 1–14.

Dietsch, G.N., Hinrichs, D.J., 1989. The role of mast cells in the elicitation of experimental allergic encephalomyelitis. J. Immunol. 142, 1476–1481.

Dietsch, G.N., Hinrichs, D.J., 1991. Mast cell proteases liberate stable encephalitogenic fragments from intact myelin. Cell. Immunol. 135, 541–548.

Dines, K.C., Powell, H.C., 1997. Mast cell interactions with the nervous system: relationship to mechanisms of disease. J. Neuropath. Exp. Neurol. 56, 627–640.

Friend, D.S., Gurish, M.F., Austen, K.F., Hunt, J., Stevens, R.L., 2000. Senescent jejunal mast cells and eosinophils in the mouse preferentially translocate to the spleen and draining lymph node, respectively, during the recovery phase of helminth infection. J. Immunol. 165, 344–352.

Galli, S., Wershil, B., 1996. The two faces of the mast cell. Nature 381, 21–22.

Galli, S.J., Tsai, M., Gordon, J.R., Geissler, E.N., Wershil, B.K., 1992. Analyzing mast cell development and function using mice carrying mutations at W/c-kit or Sl/MGF (SCF) loci. Ann. N.Y. Acad. Sci. 664, 69–88.

Ibrahim, M.Z.M., Reder, A.T., Lawand, R., Takash, W., Sallouh-Khatib, S., 1996. The mast cells of the multiple sclerosis brain. J. Neuroimmunol. 70, 131–138.

Johnson, D., Krenger, W., 1992. Interactions of mast cells with the nervous system—recent advances. Neurochem. Res. 17, 939–951.

Johnson, D., Seeldrayers, P.A., Weiner, H.L., 1988a. The role of mast cells in demyelination. 1. Myelin proteins are degraded by mast cell proteases and myelin basic protein and P2 can stimulate mast cell degranulation. Brain Res. 44, 195–198.

Johnson, D., Weiner, H.L., Seeldrayers, P.A., 1988b. Role of mast cells in peripheral nervous system demyelination. Ann. N.Y. Acad. Sci. 540, 727–731.

Lin, T.J., Issekutz, T.B., Marshall, J.S., 2000. Human mast cells transmigrate through human umbilical vein endothelial monolayers and

selectively produce IL-8 in response to stromal cell-derived factor-1 alpha. J. Immunol. 165, 211–220.

Malaviya, R.E., Ross, E., Jakschik, B.A., Abraham, S.N., 1994. Mast cell degranulation induced by type 1 fimbriated *Escherichia coli* in mice. J. Clin. Invest. 93, 1645–1653.

Mendel, I., Kerlero de Rosbo, N., Ben-Nun, A., 1995. A myelin oligodendrocyte glycoprotein peptide induces typical chronic experimental autoimmune encephalomyelitis in H-2^b mice: fine specificity and T cell receptor Vβ expression of encephalitogenic T cells. Eur. J. Immunol. 26, 1951–1959.

Nakano, T., Sonoda, T., Hayashi, C., Yamatodani, A., Kanayama, Y., Yamamura, T., Asai, H., Yonezawa, T., Kitamura, Y., Galli, S.J., 1985. Fate of bone marrow-derived cultured mast cells after intracutaneous, intraperitoneal, and intravenous transfer into genetically mast cell-deficient W/W^v mice. J. Exp. Med. 162, 1025–1043.

Neuman, J., 1890. Ueber das Vorkommen der sogneannten "Mastzellen" bei pathologischen Veraenderungen des Gehirns. Arch. Pathol. Anat. Physiol. Virchows 122, 378–381.

Nygen, H., Dahlen, G., 1981. Complement-dependent histamine release from rat peritoneal mast cells, induced by liposaccharides from *Bacteroides oralis*, *Fusobacterium nucleatum and Veillonella parvula*. J. Oral Pathol. 10, 87–94.

Orr, E.L., 1988. Presence and distribution of nervous system-associated mast cells that may modulate experimental autoimmune encephalomyelitis. Ann. N.Y. Acad. Sci. 540, 723–726.

Rodewald, H.-R., Dessing, M., Dvorak, A.M., Galli, S.J., 1996. Identification of a committed precursor for the mast cell lineage. Science 271, 819–822.

Roznieckl, J.J., Hauser, S.L., Strein, M., Lincoln, R., Theoharides, T.C., 1995. Elevated mast cell tryptase in cerebrospinal fluid of multiple sclerosis patients. Ann. Neurol. 37, 63–66.

Secor, V.H., Secor, W.E., Gutekunst, C.A., Brown, M.A., 2000. Mast cells are essential for early onset and severe disease in a murine model of multiple sclerosis. J. Exp. Med. 191, 813–822.

Seeldrayers, P.A., Yasui, D., Weiner, H.L., Johnson, D., 1989. Treatment of experimental allergic neuritis with nedocromil sodium, an anti-inflammatory drug with mast cell stabilizing properties. J. Neuroimmunol. 25, 221–226.

Silver, R., Silverman, A.J., Vitkovic, L., Lederhendler, I.I., 1996. Mast cells in the brain: evidence and functional significance. Trends Neurosci. 19, 25–31.

Steinman, L., 1996. Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. Cell 85, 299–302.

Wang, H.-W., Tedia, N., Lloyd, A.R., Wakefield, D., McNeil, H.P., 1998. Mast cell activation and migration to lymph nodes during induction of an immune response in mice. J. Clin. Invest. 102, 1617–1626.

Zhuang, X., Silverman, A.-J., Silver, R., 1997. Mast cell number and maturation in the central nervous system: influence of tissue type, location and exposure to hormones. Neuroscience 80, 1237–1245.