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Mast cell activation and neutrophil recruitment promotes early and robust inflammation in the meninges in EAE

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ABSTRACT

The meninges are often considered inert tissues that house the CSF and provide protection for the brain and spinal cord. Yet emerging data demonstrates that they are also active sites of immune responses. Furthermore, the blood–CSF barrier surrounding meningeal blood vessels, together with the blood–brain barrier (BBB), is postulated to serve as a gateway for the pathological infiltration of immune cells into the CNS in multiple sclerosis (MS). Our previous studies using mast cell-deficient ($Kit^{W/Wv}$) mice demonstrated that mast cells resident in the dura mater and pia mater exacerbate experimental autoimmune encephalomyelitis (EAE), a rodent model of MS, by facilitating CNS inflammatory cell influx. Here we examined the underlying mechanisms that mediate these effects. We demonstrate that there are dramatic alterations in immune associated gene expression in the meninges in pre-clinical disease, including those associated with mast cell and neutrophil function. Meningeal mast cells are activated within 24 h of disease induction, but do not directly compromise CNS vascular integrity. Rather, through production of TNF, mast cells elicit an early influx of neutrophils, cells known to alter vascular permeability, into the meninges. These data add to the growing evidence that inflammation in the meninges precedes CNS immune cell infiltration and establish that mast cells are among the earliest participants in these disease-initiating events. We hypothesize that mast cell-dependent neutrophil recruitment and activation in the meninges promotes early breakdown of the local BBB and CSF–blood barrier allowing initial immune cell access to the CNS.

1. Introduction

Multiple sclerosis (MS), the most common inflammatory disease of the central nervous system (CNS), affects more than 2.5 million people worldwide [for review see Ref. [1,2]]. Like many autoimmune diseases, the etiology of MS is complex. Factors implicated in susceptibility include heredity, age, gender, geographical location, and predisposing infections. This disease syndrome is characterized by perivascular inflammation in the CNS leading to demyelination of nerve fibers as well as axonal damage. The resulting interruption of motor and sensory impulses as they pass through demyelinated regions of the optic nerve, brain or spinal cord results in numerous and varied neurological deficits.

Although the CNS is the major site of immune-mediated damage in MS, there is substantial evidence that inflammation in the meninges, multipartite membranes covering the brain and spinal cord, is critical for disease pathogenesis. The meninges are comprised of three distinct tissue layers: the outermost dura mater, the arachnoid mater and the innermost pia mater. The arachnoid mater and the pia mater are collectively referred to as the leptomeninges and enclose the cerebral spinal fluid (CSF) that flows through the subarachnoid space [3–6]. Conventional wisdom asserts that myelin-specific CD4+ Th1 and Th17 cells, the major orchestrators of CNS damage, are initially activated in peripheral lymphoid organs and only upon gaining access to the CNS through the normally impermeable blood–brain barrier (BBB) are these cells reactivated to initiate local inflammation

Abbreviations: CFA, complete Freund's adjuvant; PTX, pertussis toxin; DC, dendritic cell; BMMC, bone marrow mast cells; BBB, blood brain barrier; CSF, cerebral spinal fluid; CNS, central nervous system; MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; qRT-PCR, quantitative real-time PCR; i.c., intracranial; i.p., intraperitoneal.

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[reviewed in Ref. [7]]. Data from studies in both MS and experimental autoimmune encephalomyelitis (EAE), the rodent model of MS, supports an alternative model in which early T cell reactivation in the meninges precedes large scale entry of inflammatory cells into the CNS. Using intravital microscopy, myelin-reactive T cells can be detected migrating from the periphery and entering the meninges prior to accessing the CNS parenchyma in EAE. Furthermore their interaction with APCs in the subarachnoid space induces T cell proliferation [8–11]. Myelin antigens, which normally drain from the CSF into the meninges and cervical lymph nodes, can be observed co-localizing with MHC Class II^+ DCs and macrophages at these sites [12]. "Inflamed meninges", characterized by dense T cell infiltrates and/or ectopic B cell follicles, have also been identified post-mortem in patients with progressive MS and are often associated with diffuse axonal loss and cortical degeneration [13,15,16]. A recent study by Lucchinetti et al. identified leptomeningeal B cell aggregates and cortical demyelination in early stage MS patients suggesting that the formation of tertiary lymphoid tissue is not restricted to late stage disease [17]. There is also evidence of ectopic lymphoid organ formation in the meninges in EAE models of both relapsing–remitting and chronic disease [14,18,19]. However, as in MS, their direct contribution to EAE has yet to be definitively established.

DCs and macrophages reside in the meninges even in the absence of inflammation [10]. However, it is less appreciated that mast cells, best known for their role in allergic responses, are also normal residents [20–23]. Mast cells, c-kit+ FcεRI+ innate immune cells, reside in most tissues and are often located in close association with blood vessels and nerves. These cells are considered first-line responders to infectious insults due to their prevalence in areas that interface with the external environment including the skin and the mucosa of genitourinary, respiratory and gastrointestinal tracts [reviewed in Ref. [24,25]]. Activation of mast cells is achieved through receptors that engage pathogen- and danger- associated molecular pattern molecules (PAMPs and DAMPs), complement components, hormones and cytokines as well as through cross-linking of IgE and IgG Fc receptors. These distinct activation modes can result in the rapid release of pre-formed mediators in granules and the *de novo* synthesis of multiple potent pro- and anti-inflammatory mediators that can regulate vascular permeability, cell recruitment and cell activation [reviewed in Ref. [26–29]]. Recent data from our laboratory indicates that meningeal mast cells play an essential role in promoting severe EAE. Mast cell-deficient mice (WBB6 $Kit^{W/Wv}$: W/W^v) exhibit a mild disease course that is associated with significantly decreased immune cell infiltration to the CNS compared to wild type littermates and a failure to breach the BBB, an index of CNS vascular integrity [23,30]. Selective reconstitution of the meningeal mast cells to $Kit^{W/Wv}$ mice via intracranial (i.c.) injection of bone marrow-derived mast cells (BMMCs) restores the wild type disease phenotype [23]. Reconstitution of the meninges with $TNF^{-/-}$ mast cells fails to restore severe disease indicating TNF is essential for mediating mast cell effects.

In this study we examined immune activity in the meninges in pre-clinical disease in wild type and $Kit^{W/Wv}$ mice to determine how mast cells and c-kit signaling pathways exert an influence. Microarray analyses of calvarial meningeal tissues isolated from naïve mice and immunized mice at day 6 post-active disease induction reveal striking alterations in the expression of many immune response genes, some of which are c-kit dependent. We also show evidence of mast cell activation in the meninges within 24 h post-immunization that is coincident with neutrophil recruitment to these sites, events dependent on mast cell-derived TNF expression. These data establish that mast cells are among the earliest participants in EAE-initiating events and act to promote meningeal inflammation through the recruitment of neutrophils, cells previously shown to be necessary for loss of BBB integrity [31]. We hypothesize that these early events set the stage for the loss of local vascular integrity that leads to pathological immune cell influx into the CNS.

2. Methods

2.1. Mice

Female $Kit^{W/Wv}$ mice (WBB6F1/J-Kit^W/Kit^{Wv}), wild type littermate controls, $TNF^{-/-}$ mice (on the C57BL/6 background) as well as C57BL/6 mice were purchased from Jackson Laboratories at 3–5 weeks of age and housed in the barrier facility at Northwestern University. $HDC^{-/-}$ mice (on the C57BL/6 background) were originally generated by Dr. Hiroshi Ohtsu (Tohoku University) and were the kind gift of Dr. Paul Bryce (Northwestern University) [32]. All experiments were approved by the Northwestern University Animal Care Committee and all mice were housed in an Association for Assessment and Accreditation of Laboratory Animal Care–approved facility.

2.2. Induction of chronic experimental autoimmune encephalomyelitis

Mice were immunized subcutaneously with 100 μg $MOG_{35–55}$ peptide emulsified in 5 mg/mL CFA (Incomplete Freund's Adjuvant with dessicated M. Tuberculosis H37 RA, VWR). On Days 0 and 2, animals received i.p. injections of 250 ng pertussis toxin (List Biologicals.) Disease severity was assessed every other day beginning on Day 10, and assigned a value using the following scale: 0, no disease; 1, tail flaccidity; 2, hind limb weakness; 3, hind limb paralysis; 4, hind limb paralysis with muscle wasting and inability to right from supine; 5, moribund.

2.3. Blood–brain barrier permeability assay

Naïve or immunized mice were injected i.p. with 10% sodium fluorescein (NaFlu) (Sigma Aldrich). After 10 min blood was drawn and the mice were perfused with PBS through the left cardiac ventricle. Spinal cords were removed, weighed, homogenized and processed along with cardiac blood for NaFlu content. Fluorescence was measured by a SpectraMax Gemini XS fluorimeter and presence of NaFlu in the samples was quantified using a standard curve. The uptake of NaFlu into the CNS was calculated using the following equation: (NaFlu in spinal cord (mg)/weight of spinal cord (mg/mL))/(NaFlu in cardiac blood (mg)/amount of cardiac blood (μl)).

2.4. Reconstitution of mast cell-deficient mice with bone marrow-derived mast cells

Bone marrow was harvested from femurs of mice and cultured in complete RPMI with recombinant murine IL-3 and stem cell factor as described [30]. BMMC were used after a minimum of 6 weeks in culture at >96% purity as determined by flow cytometric analysis verifying a homogenous population of c-kit^{hi} FcεRI+ cells. To establish reconstitution, BMMC were injected i.c. at a concentration of 1×10^6 BMMC in 50 ul of PBS.