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Immunopathology of Japanese macaque encephalomyelitis is similar to multiple sclerosis

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ABSTRACT

Japanese macaque encephalomyelitis (JME) is an inflammatory demyelinating disease that occurs spontaneously in a colony of Japanese macaques (JM) at the Oregon National Primate Research Center. Animals with JME display clinical signs resembling multiple sclerosis (MS), and magnetic resonance imaging reveals multiple T_2-weighted hyperintensities and gadolinium-enhancing lesions in the central nervous system (CNS). Here we undertook studies to determine if JME possesses features of an immune-mediated disease in the CNS. Comparable to MS, the CNS of animals with JME contain active lesions positive for IL-17, CD4 + T cells with Th1 and Th17 phenotypes, CD8 + T cells, and positive CSF findings.

1. Introduction

Multiple sclerosis (MS) is widely believed to be an immune-mediated disease that leads to multifocal destruction of the myelin and to a lesser extent axonal degeneration. It is proposed that MS pathogenesis is driven by auto-reactive T cells that aberrantly gain access to the central nervous system (CNS). Upon entry to the CNS, these T cells become reactivated when they recognize components of myelin, setting in motion a cascade of inflammatory events that ultimately lead to demyelination and axonal injury (Goverman, 2009). The mechanisms that trigger these T cells to become pathogenic are poorly understood, but genetic and environmental triggers are thought to play an important role (International Multiple Sclerosis Genetics et al., 2011). T cells expressing the cytokines IL-17 or IFN-γ appear to be key players in disease development, as studies have shown that these populations are present in MS lesions (Kebir et al., 2009, 2007; Tzartos et al., 2008). This highlights a central role for helper T cells (Th) in MS progression, and emphasizes the need to understand the function of these T cell subsets in order to further unravel the etiology of the disease (Waisman et al., 2015). Active MS lesions also contain CD8 + T cells, macrophages filled with myelin debris and reactive astrocytes (Popescu and Lucchinetti, 2012). Beyond the CNS, groups have reported an increased prevalence of auto-reactive myelin-specific T cells in the peripheral blood of MS patients (Kerlero de Rosbo et al., 1993; Hedegaard et al., 2008). However, conflicting results have been published showing that both healthy controls (HC) and MS patients have similar frequencies of these T cells in the periphery (Hellings et al., 2001, 2002). These data hint at the role of defective regulation in controlling these potentially pathogenic T cells in MS patients as compared to HC.

B cells and plasma cells also play a significant role in MS immunopathogenesis, as their products are frequently detected in MS lesions and cerebrospinal fluid (CSF) (Berer et al., 2011; Probstel et al.,

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2015; Serafini et al., 2004). Importantly, the detection of intrathecally synthesized oligoclonal IgG bands (OCBs) in the CSF is a commonly used paraclinical measure to diagnose MS when utilized in conjunction with MRI and clinical history (Petzold, 2013; Bonnan, 2015). Further substantiating the role of B cells in MS are clinical trials with the anti-CD20 monoclonal antibodies, rituximab and ocrelizumab, a treatment that resulted in reduced inflammatory brain lesions as detected by MRI and decreased clinical relapses in subjects with relapsing MS (Hauser et al., 2008).

Animal models that mimic the immune-mediated aspects of MS are valuable in elucidating mechanisms associated with disease pathogenesis. The most widely utilized animal model to study MS is experimental autoimmune encephalomyelitis (EAE). EAE can be induced in a variety of species, including mice, rats and nonhuman primates (NHP). EAE induction involves immunizing animals with myelin proteins or peptides in Freund's complete adjuvant and results in T cell responses to myelin, focal inflammatory lesions within the CNS and ultimately leads to paralysis. EAE recapitulates the T cell-mediated aspects of MS, as studies find Th1 and Th17 cells are necessary for the induction of EAE (Jager et al., 2009). However, while EAE studies have yielded useful insight into several facets of MS pathogenesis, this model has well recognized limitations. First, immunizing animals with myelin proteins or peptides artificially induces the disease EAE, while MS occurs as a spontaneous disease. Second, EAE is studied in inbred mouse strains and this is in large contrast to MS, which occurs, in a heterogeneous population with highly variable genetic diversity. Third, the relatively small size of mice constrains the imaging that can be performed using MRI, whereas an array of sophisticated MRI techniques are available to study MS. An animal model that overcomes these limitations would be of considerable use in advancing our understanding of MS.

In 2011, we described a spontaneous inflammatory CNS demyelinating disease, called Japanese macaque encephalomyelitis (JME), that occurs in 1% to 3% of the animals in our colony of Japanese macaques (JM, *Macaca fuscata*) at the Oregon National Primate Research Center (ONPRC) (Axthelm et al., 2011). We proposed that JME was a spontaneous NHP model for MS based on the appearance of brain lesions as detectable with MRI and the presence of multifocal demyelinating lesions as observed using histology. Here, we present evidence that JME shares many immunopathological similarities with MS. Specifically, we demonstrate that JME lesions possess similar immunohistopathological features as MS lesions, including active demyelination and significant T- and B-cell germinal areas surrounding perivascular and periventricular spaces. Importantly, immunofluorescence analysis revealed that within demyelinating lesions, both astrocytes and oligodendrocytes stain positively for interleukin 17 (IL-17). Moreover, multicolor flow cytometry analysis of infiltrating T cells revealed both $CD4^+$ and $CD8^+$ T cells that expressed IL-17 or IFN-γ, and in some instances both cytokines, similar to what has been shown in MS. Finally, we show that animals with JME have positive CSF findings that include an elevated IgG index and 2 or more oligoclonal bands, a common finding in MS (Polman et al., 2011). Collectively, these data support JME as a unique NHP model for a MS-like disease, which can be utilized to offer new insights into the pathogenesis of MS.

2. Materials and methods

2.1. Animals and animal procedures

All animal protocols and procedures were reviewed and approved by the ONPRC Institutional Animal Care and Use Committee. The ONPRC is an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accredited research facility and conforms to National Institutes of Health guidelines on the ethical use of animals in research. JM exhibiting neurological dysfunction and symptoms associated with JME were brought in for physical examination and provided supportive care, and then scanned by MRI on a 3 T Siemens TIM Trio MR instrument as previously described (Axthelm et al., 2011). Animals with progressive disease were humanely euthanized via exsanguination, with blood and CSF collection. The blood was immediately processed for plasma collection and peripheral blood mononuclear cell (PBMC) isolation. The CSF and serum were archived at −80 °C, while PBMCs were cryopreserved. Animals were subsequently perfused with sterile PBS through carotid cannulation, the brain was removed and immersed fixed in 4% buffered paraformaldehyde solution. For some necropsies, lesion areas that were detected by MRI were collected from affected areas after the PBS perfusion step in order to obtain fresh brain tissue for lymphocyte analysis. A portion of each lesion was placed in RPMI media supplemented with 10% fetal bovine serum and processed as described to isolate CNS-infiltrating mononuclear cells (CNS-MNCs) (Jager et al., 2009). In these cases the remainder of the brain was also immersed fixed in 4% neutral buffered paraformaldehyde solution for histopathological analysis. For comparisons to JME cases, blood and cisternal cerebrospinal fluid (CSF) samples were also collected from healthy controls (HC) after sedation with Telazol during routine physical examinations, and then processed as above. Each HC was subsequently given Carprofen (4 mg/kg) subcutaneously to minimize discomfort.

2.2. Histopathological examination

Fixed tissue was processed for paraffin embedding and subsequent histology. Fixed sections (0.5 mM) from the CNS were prepared and stained with Luxol fast blue (LFB) and hematoxylin and eosin (H&E) to visualize demyelinating regions and infiltrating inflammatory cells. Sections containing lesions were treated essentially as described previously (Axthelm et al., 2011). For antigen detection analysis, the sections were deparaffinized, blocked with 10% goat serum and 5% bovine serum albumin, and endogenous peroxidase was quenched by standard techniques. Slides were treated with primary antibodies overnight at 4 °C and then processed for color detection or immunofluorescence. Commercially available primary antibodies were used to detect the following cellular antigens and cell types: myelin basic protein (MBP, mouse anti-MBP, clone SMI 99; Covance, Princeton, NJ, 1:500), glial fibrillary acidic protein (GFAP, rabbit polyclonal anti-GFAP, RPCA-GFAP, EnCor Biotechnology, Gainsville, FL, 1:500), oligodendrocytes (rabbit anti-olig-2, AB9610, Millipore, Billerica, MA, 1:200), activated microglia (rabbit anti-IBA-1, 019–19,741, Wako, Richmond, VA, 1:300), macrophages (mouse anti-human CD163, clone EDHu-1, AbD Serotec, Hercules, CA, 1:100), T cells (rabbit anti-human CD3, Dako, Carpinteria, CA, 1:200), B cells (mouse anti-CD20, clone L26, Dako, 1:200), activated complement C3d (rabbit polyclonal anti-3Cd, AB15981, Abcam, Cambridge, MA, 1:400) and IL-17 (mouse anti-IL17 F, clone 4H1629.1 Rockland, MD, 1:100).

For immunohistochemistry, detection of biotinylated secondary antibodies was performed with peroxidase ABC (Elite kit, Vector Laboratories, San Carlos, CA) and visualization with DAB (Dako) for CD163 and activated complement 3Cd, and Vector SG for MBP (Vector Laboratories). For immunofluorescence detection, secondary biotinylated goat anti-mouse (Vector Laboratories) or goat anti-rabbit IgG (H + L) (Vector Laboratories) were used, followed by Elite ABC kit and further stained with streptavidin Alexa 488 (Molecular Probes, Eugene, OR) and streptavidin Alexa 594 (Molecular Probes), respectively, to visualize the antigens of interest. The sections were counterstained with 4,6′-diamino-2-phenylindole dihydrochloride (DAPI) (1:5000) and covered with Prolong gold anti-fade medium (Invitrogen, Carlsbad, CA) or Omnimount (National Diagnostics, Atlanta, Georgia). Sections were examined using a Zeiss Axio Imager M1 microscope (Carl Zeiss, Thornwood, NY) using Plan NeoFluar objective lenses (2.5×/0.5 NA and 40×/0.75 NA). Optical images were obtained with standard