Regulation of Matrix Metalloproteinase (MMP) and Tissue Inhibitor of Matrix Metalloproteinase (TIMP) Genes During JHMV Infection of the Central Nervous System

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1. INTRODUCTION

The central nervous system (CNS) is refractive to many aspects of the immune system primarily due to its limited ability to repair damage induced by the cytopathic mechanisms deployed by most immune cells. A primary obstacle to CNS inflammation is the blood-brain-barrier (BBB), which limits entry of immune cells into the CNS. To pass the BBB, inflammatory cells release matrix metalloproteinases (MMPs); a growing family of proteases with overlapping substrate specificities for components of the extracellular matrix (reveiwed by Kieseier et al. 1999). MMPs break down the extracellular matrix surrounding the endothelial layer of BBB thereby permitting peripheral immune cells to traverse the BBB and migrate through the parenchyma of the CNS in response to inflammatory signals. To limit potential damage resulting from infiltration of inflammatory cells, MMP activity is tightly regulated at both the level of gene expression and proenzyme activation as well as by expression of a second gene family, the tissue inhibitors of MMPs (TIMPs). TIMPs act as competitive inhibitors for the active sites of MMPs and thus limit inflammatory infiltrates.

The Nidoviruses (Coronaviruses and Arteriviruses).
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To evaluate the role of individual MMPs and TIMPS in facilitating mononuclear cell infiltration during acute CNS viral infection, male BALB/c mice were infected with JHMV, a neurotropic strain of mouse hepatitis virus. JHMV induces lesions similar to those associated with the human disease multiple sclerosis and pathology is associated with extensive infiltration by NK cells, $CD4^+$ and $CD8^+$ T cells and peripheral macrophages. These inflammatory cells appear as early as day 5 to 6 post infection (p.i.) and peak by day 7 p.i. (Williamson et al. 1991) Naïve and JHMV infected mice were assessed for MMP and TIMP gene expression by means of a RNAse protection assay (RPA). Results from RPA revealed that genes encoding MMP-2, -9, -10 and -14 as well as TIMP-2 and -3 were already expressed within the uninfected CNS, however their expression remained unchanged in response to JHMV infection. By contrast MMP-3 and -12, which were not detected in naïve mice, were rapidly induced following JHMV infection. TIMP-1 was similarly induced within the CNS of JHMV infected mice. These data suggest that MMP-3 and -12 may be involved in immune cell infiltration of the CNS and that their activity may be limited by expression of TIMP-1 during acute JHMV expression.

2. MATERIALS AND METHODS

Animals and virus. Male BALB/c mice were purchased from National Cancer Institute and used between 6 to 8 weeks of age. Mice were infected with 1000 PFU of the 2.2-V-1 strain of JHMV (Fleming et al. 1986).

RNA isolation and RPA. Total RNA was prepared from brains of naïve and JHMV infected mice using TRIzol reagent in accordance with the manufacturer's protocol. Plasmids containing MMP and TIMP RPA probes were a gift from Iain Campbell (Pagenstecher et al. 1997). 10 μg of total brain RNA was hybridized with α-^{32}p labeled MMP or TIMP probe sets and RPA were performed as described (Pagenstecher et al. 1997). Protected probe fragments were separated on a 5% acrylamide/7 M urea denaturing gel. Intensities of bands were quantitated using a Molecular Dynamics phosphorimager.

3. RESULTS

Infection with the neurotropic strain of JHMV results in extensive infiltration by peripheral mononuclear cells. To identify the role of individual MMPs involved in mononuclear cell infiltration of the CNS, a multi-probe RPA was used to assay MMP mRNA expression during acute

JHMV infection. Total RNA prepared from the brains of both naïve and infected mice sacrificed at the indicated time points was hybridized with a probe set specific for MMP-2, -3 and -9 (Fig. 1A). Both the MMP-2 and -9 genes were expressed on a constituative basis, with no apparent differences between naïve and infected mice. However, induction of MMP-3 expression, which was not detectable within the CNS of naïve mice, was induced quite rapidly following JHMV infection and was observed as early as day 2 p.i. MMP-3 expression was transient, however, peaking at day 4 p.i. and was not detected after day 10 p.i.

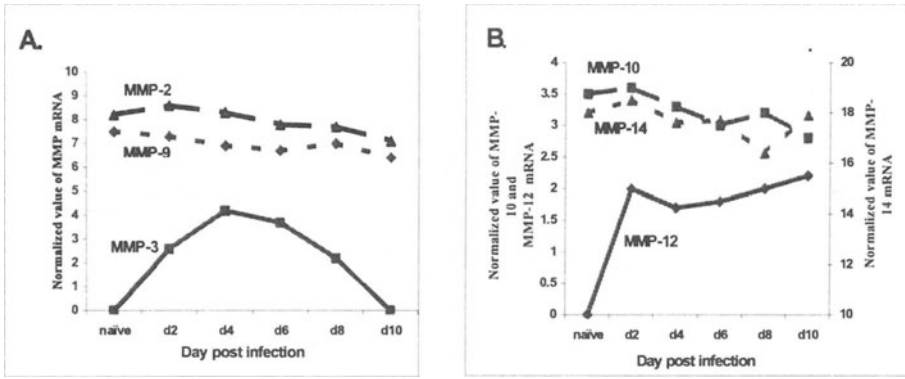

Figure 1. Expression of MMP RNA during acute JHMV infection. RPA were performed to determine the relative expression of MMP-2, -3 and -9 mRNA (Panel A) and MMP-10, -12 and -14 mRNA (Panel B) within brains of naïve or JHMV infected mice sacrificed at the indicated time points.

A second probe set was used to examine expression of MMP-10, -12 and -14 (Fig. 1B). MMP-14 mRNA (more commonly referred to as membrane type MMP-1 [MT1-MMP]), was expressed at a very high level compared to expression of other MMP genes, possibly due to its role as an activator of secreted MMP proenzymes. However, MMP-14 expression was constitutive and no induction of mRNA was observed following acute JHMV infection. Similarly, MMP-10 was expressed within the CNS of naïve mice, but was not induced within the CNS of JHMV infected mice. MMP-12 expression was not observed within the CNS of naïve mice, but was detected as early as day 2 p.i. in the brains of JHMV infected mice. In contrast to the transient expression of MMP-3, however, MMP-12 expression remained elevated after clearance of infectious virus and was detected as late as day 30 p.i.

TIMPs are a family of specific regulatory inhibitors of MMP activity and are critical for limiting the extent of immune cell infiltration. To determine whether expression of TIMPs were altered by JHMV infection, total brain

RNA was hybridized with a RPA probe set consisting of TIMP-1, -2 and -3 (Fig. 2). TIMP-1 mRNA was barely detectable in naïve mice, but expression was rapidly upregulated following JHMV infection. TIMP-1 mRNA expression peaked at day 6 p.i. and remained elevated compared to naïve mice as late as day 30 p.i. By contrast expression of TIMP genes 2 and 3, although detectable within the brains of naïve mice, was not altered by JHMV infection.

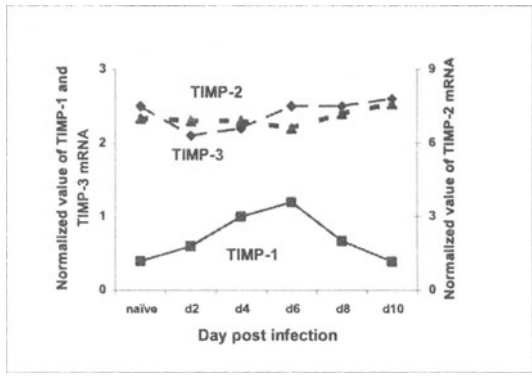

Figure 2. TIMP mRNA expression during acute JHMV infection. RPA were performed to determine the relative expression of TIMP-1, -2, and -3 mRNA within brains of naïve or JHMV infected mice sacrificed at the indicated time points.

4. DISCUSSION

In order to elucidate the role of MMPs during CNS inflammation, MMP and TIMP gene expression was examined following acute JHMV infection. mRNA expression from the MMP-2, -9, -10 and -14 genes was detected in the brain of naïve BALB/c mice. However, no alterations in mRNA expression were observed from any of these genes following intracerebral JHMV infection. Expression of these MMPs within the CNS of naïve mice and subsequent lack of change in expression during inflammation suggests that these genes may be involved in normal remodeling of the extracellular matrix within CNS.

It has been reported that MMP-2 and -9 are the predominant MMPs produced by T cells upon binding with adhesion molecules (Esparza et al. 1999, Goetzl et al. 1996). Surprisingly, mRNA expression of both MMP-2 and –9, which were detected in the CNS of naïve mice, were not induced by JHMV infection despite extensive T cell infiltration (data not shown). Although, posttranscriptional regulation of these proteins cannot be ruled out, it appears likely that other MMPs may be required to make a

contribution towards permeation of the extracellular matrix of the BBB during acute JHMV infection. MMP-3 and -12, which were not detected within the CNS of naïve mice, were both induced within 48 h of JHMV infection. MMP-3 was expressed in a transient fashion, whereas MMP-12 remained elevated following infection with JHMV. MMP-3 may be involved in the breakdown of the BBB as it is specific for several components of the BBB basal lamina. Identifying the role of MMP-12, which is an elastase, may be more problematic, although elastin has been shown to account for up to 4% of the ECM of brain microvessels (Faris et al. 1982). These data suggest that MMP-3 and –12, which have been linked to expression by mononuclear cells (Ozenci et al. 1999, Shapiro et al. 1999, Maeda and Sobel 1996) and are also up-regulated during EAE (Pagenstecher et al. 1998) may play an active role during mononuclear cell infiltration in response to CNS infection.

Examination of TIMP mRNA levels revealed that TIMP-2 and -3 are expressed in naïve mice and that expression remains constitutive following JHMV infection. By contrast, TIMP-1 expression was induced several fold following JHMV infection. These data on TIMP expression following JHMV infection are a quite similar to those reported for mice following induction of EAE (Pagenstecher et al. 1998) and suggest that TIMP-1 may be the primary inhibitor of MMPs released during either viral or autoimmune induced inflammation. To be noted, simultaneous up-regulation of MMP-3 and TIMP-1 in the JHMV model is consistent with the previous reports that TIMP-1 can form complexes with MMP-3 *in vitro* (Gomis-Ruth et al. 1997), suggesting specific inhibition of MMP-3 by TIMP-1. Studies of *in vitro* cultured cells and murine models of neuropathogenesis suggests that astrocytes may be a primary source of TIMP-1 (Giraudon et al. 1998 Pagenstecher et al. 1998). Taken together these data suggest that T cells and microglia/macrophages may function in a proinflamatory role through release of MMP-3 and -12 and that this action is controlled by expression of TIMP-1, possibly secreted by astrocytes.

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