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Review

Pathogenic Immunity in Theiler's Virus-Induced Demyelinating Disease: a Viral Model for Multiple Sclerosis

BYUNG S. KIM[1]*, JOANN P. PALMA[1], ATSUSHI INOUE[2] and CHANG-SUNG KOH[2]

[1] Departments of Microbiology-Immunology and Pathology, Northwestern University Medical School, Chicago, IL 60611, USA, [2] Third Department of Internal Medicine, Shinshu University School of Medicine, Matsumoto 390, Japan

Abstract. Multiple sclerosis involves inflammatory immune responses in the central nervous system (CNS) and is considered as an autoimmune disease potentially associated with viral infection. The majority of experimental models rely heavily on the autoimmune components since similar diseases can be induced following immunization with various myelin antigens. A very attractive alternative model is the Theiler's murine encephalomyelitis virus-induced demyelinating disease. This disease is primarily a $CD4^+$ T cell-mediated, inflammatory demyelinating disease induced following viral infection. Virus-specific inflammatory Th1 cell responses, rather than cytotoxic T lymphocyte response, play a critical role in the pathogenic immune responses. The major pathogenic epitopes have been identified and these are correlated with a Th1 type response to the epitopes following viral infection. In addition, the initial virus-specific immune response is followed by the autoimmune responses to myelin antigens. Assessment of cytokines produced locally in the CNS during the course of disease suggests involvement of inflammatory cytokines in the disease. Furthermore, the manipulation of inflammatory cytokine levels by administration of either recombinant cytokines or antibodies to the cytokines strongly influences the induction and/or progression of disease, supporting the importance of these inflammatory cytokines in this virus-induced demyelinating disease.

Key words: multiple sclerosis; Theiler's virus; demyelinating disease.

Introduction

Multiple sclerosis (MS) is a neurological disease characterized by demyelination in the white matter of the brain and spinal cord mediated by inflammatory immune responses[1]. The clinical symptoms range from a mild nervous system disability to a severe degenerative paralyzing disorder. Because of the relatively high incidence and chronic nature of the disease, social and economic loss due to it is enormous. Although the cause of MS is unknown, one or more infectious agents may be involved in the initial tissue damage leading to autoimmunity[18, 27, 46]. Several virus-induced and autoimmune models have been used to study the pathogenic mechanisms of this disease[2, 7, 8, 28]. Among these experimental model systems, Theiler's murine encephalomyelitis virus (TMEV)-induced demyelination provides an excellent infectious model for MS[7, 28].

Abbreviations used: MS – multiple sclerosis, TMEV – Theiler's murine encephalomyelitis virus, CNS – central nervous system, Th – helper T lymphocytes, CTL – cytotoxic T lymphocytes, MHC – major histocompatibility complex, IDD – immune-mediated demyelinating disease, LPS – lipopolysaccharide.

* Correspondence to: Dr. Byung S. Kim, Department of Microbiology-Immunology, Northwestern University Medical School, 303 E. Chicago Ave., Chicago, IL 60611, USA, tel.: +1 312 503 86 93, fax: +1 312 503 13 39, e-mail: bskim@nwu.edu

TMEV is a common enteric pathogen in mice and belongs to the *Picornaviridae*[24, 48]. The TMEV genome is encapsulated in an icosahedral capsid structure composed of 60 units of four capsid proteins (VP1, VP2, VP3 and VP4)[24, 48]. There are two major subgroups of TMEV: the GDVII and FA, viruses causing rapid fatal encephalitis, and the BeAn and DA strains, which result in a biphasic neurological disease in susceptible mice[7]. An intracerebral inoculation of TMEV DA or BeAn into susceptible mice results in a chronic immune-mediated demyelinating disease (IDD) that shares many of the features of human MS. Although undetectable with BeAn, the early acute phase of infection with DA displays flaccid limb paralysis and degeneration of neurons. On the other hand, a chronic inflammatory demyelination is characteristic of the late phase disease in both DA and BeAn infections. In addition, demyelination is primarily associated with cell-mediated immune responses and strong autoimmunity to myelin antigens is induced following the initial demyelination by virus-specific T cells[28].

Pathogenic Effect of $CD4^+$ T Cell Responses

Treatment with antibodies to either the class II or CD4 molecules suppressed demyelination induced by TMEV[41, 50], strongly suggesting that the Th cell response is involved in the pathogenesis of demyelination. Subcutaneous immunization with UV-inactivated TMEV prior to, but not after, viral infection efficiently protects susceptible mice from demyelinating disease[53]. The immune components involved in the protection/pathogenesis have been further analyzed by utilizing various fusion proteins containing individual viral capsid proteins or synthetic peptides representing antibody and/or T cell epitope regions. The locations of the major Th epitopes and linear antibody epitopes are diagrammatically shown in Fig. 1.

The majority of MHC class II-restricted helper T cell lines/clones derived from demyelinating lesions of the spinal cords after viral infection react with VP1 or VP2 fusion protein, suggesting that these virus-specific Th cells in the CNS are involved in the pathogenicity[56]. In addition, the majority of T cells (~90%) appear to recognize one of three predominant epitopes ($VP1_{233-250}$, $VP2_{74-86}$ and $VP3_{24-37}$), one on each major external capsid protein[55]. It is interesting to note that the VP3 Th epitope overlaps with the major VP3 linear antibody epitope (Fig. 1). Although the T cell responses to the three predominant epitopes are similar throughout the disease course, the level of IFN-γ production was highest in response to VP1, intermediate with VP2 and lowest with the VP3 epitope peptide[57]. Conversely, the production of Th2 cytokines is highest in response to VP3 and lowest following stimulation with VP1 epitope peptides. Preferential induction of the Th2 response by the VP3 epitope may be influenced by the overlap with a major antibody epitope[57]. Further immunization with the epitope peptides indicated that the $VP1_{233-250}$ and $VP2_{74-86}$ epitopes exacerbate the disease while the $VP3_{24-37}$ epitope does not. Moreover, most of the T cell clones derived from affected spinal cords produced IL-2 and IFN-γ, but not IL-4, suggesting that the majority of Th cells in the CNS is Th1[33, 56, 57]. In addition, the production of Th1 cytokine messages in the CNS preceded that of Th2 cytokine messages in virus-infected SJL mice, suggesting that the initial establishment of a Th1 response is critical for the pathogenesis of TMEV-induced demyelination. Taken together, these experiments strongly suggest that $CD4^+$ helper T cells are primarily involved in the pathogenesis of TMEV-IDD, in particular in inflammatory Th1 responses to the VP1 and VP2 epitopes.

A spontaneous non-pathogenic variant virus displayed a single substitution of lysine to arginine at position 244 within the $VP1_{233-250}$ epitope (Fig. 1), which is the only amino acid difference found in the P1 region encoding all of the capsid proteins[20]. The overall T cell response to the variant virus is primarily Th2 type, whereas that induced after infection with the wild type is Th1 type (unpublished data). Thus, such a spontaneous single amino acid change in a predominant Th epitope of a viral coat protein may be able to exert

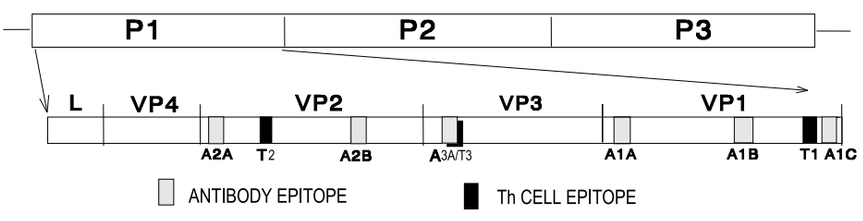

Fig. 1. Viral genomic structure and the major epitopes for antibody as well as Th cells are schematically shown. VP1, VP2 and VP3 of Theiler's virus are external capsid proteins. Six major linear antibody epitopes and three Th epitopes have been found. These three Th epitopes, one on each external capsid protein, represent the great majority (~90%) of Th responses to TMEV

a profound impact on the type of Th response determining the pathogenicity of a virus, hence altering the outcome of disease.

The T cell repertoire involved in the recognition of the representative pathogenic epitope, $VP1_{233-250}$, appears to be very diverse[19]: fine epitope regions recognized by individual hybridomas are broad and largely overlapping, but not identical. Although close to 50% of the T cell hybridomas reactive to $VP1_{233-250}$ used the T cell receptor Vβ16, the CDR3 region was extremely heterogeneous among the T cell clones. However, Vβ16 (but not Vα) appears to be associated with recognition of the C-terminal residue of the minimal T cell epitope. Nevertheless, the broad diversity and complexity of the T cell repertoire towards the predominant viral determinants on capsid proteins are apparent despite the limited number of epitope regions involved in T cell recognition.

Protective Role of $CD8^+$ T Cell Responses

The potential role of $CD8^+$ T cells in the pathogenesis of demyelination has been controversial. A group of investigators proposed that cytotoxic T cells (CTL) specific for virus-infected cells are necessary for clinical manifestation. This is based on the observation that CTL-defective, perforin-deficient C57BL/6 mice do not develop clinical symptoms[30, 40]. However, this conclusion could be misleading because these mice are capable of developing clinical symptoms, especially following immunization with UV-inactivated virus (unpublished observation). Moreover, treatment of susceptible mice with anti-CD8 antibodies does not alter the course of TMEV-induced demyelinating disease[4]. We and others have shown that viral infection can lead to clinical symptoms even in β_2-microglobulin-deficient resistant mice lacking functional $CD8^+$ T cells[12, 36]. In addition, β_2-microglobulin-deficient susceptible SJL mice also develop demyelinating disease (unpublished observation). These results strongly suggest that virus-specific CTLs are not likely involved in the pathogenesis of demyelination. Recently, it has also been demonstrated that a high level of CTLs is induced in resistant mice and a low level in susceptible mice[9]. Thus, the inconsistent level of virus-specific CTLs in susceptible SJL mice[22, 23] may reflect a poor $CD8^+$ T cell response, indicating a protective role of $CD8^+$ cytotoxic T cells, perhaps by limiting virus-infected cells. Adoptive transfer of $CD8^+$ T cells confers resistance to TMEV-induced demyelination in susceptible recipient mice[31], again consistent with a protective role of $CD8^+$ CTLs. Therefore, the major role of virus-specific CTLs is most likely for protection rather than tissue damage leading to clinical symptoms.

Lessons Learned from Cytokine Manipulation

Several investigators further explored the potential role of proinflammatory cytokines in the virus-induced pathogenesis of demyelination (Table 1). Since bacterial lipopolysaccharide (LPS) produced by Gram negative bacteria and IL-1 are known to potentiate an immune response and trigger the production of various inflammatory cytokines, the effects of LPS or IL-1 on TMEV-induced demyelinating disease were assessed[38]. Intraperitoneal injection of LPS or IL-1β into genetically resistant C57BL/6 mice, concomitant with viral infection, resulted in clinical symptoms in approximately 50% of the group compared to 0% in control mice. The increase in susceptibility following LPS-treatment

Table 1. Effects of cytokines or antibodies to various cytokines on Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD)

Cytokines or antibodies	Effects on TMEV-IDD	References
IL-1/ LPS	Induces demyelinating disease in resistant mice	38
	Renders low-pathogenic virus pathogenic	33
TNF-α/anti-TNF-α	rTNF-α inhibits the level of demyelination	35
	Anti-TNFα inhibits demyelinating disease	15
	Inhibitors for TNF-α suppress development and progression of demyelinating disease	14, 54
IL-12/anti-IL-12	Anti-IL-12 inhibits demyelinating disease	16
	Anti-IL-12 does not inhibit demyelination	5
IFN-γ/anti-IFN-γ	IFN-γ and anti-IFN-γ exacerbate demyelinating disease	37
	Anti-IFN-γ accelerate demyelination	42
	IFN-γ receptor deficiency converts resistant mice to susceptible	13
iNOS	Antagonists inhibit development and progression of demyelinating disease and inflammatory responses	17,43

iNOS – inducible nitric oxide synthase.

correlated with enhanced levels of TMEV-specific Th1 response as measured by delayed type hypersensitivity and proliferation. Similar LPS treatment of susceptible SJL/J mice, after infection with a low-pathogenic variant of TMEV, resulted in 80–100% of the mice developing clinical symptoms, while none of the control mice were affected clinically[33]. These results strongly suggest that an increase in the general inflammatory response is capable of promoting a virus-specific Th1 response and inducing demyelinating disease in resistant mice infected with pathogenic virus or susceptible mice infected with a non-pathogenic virus.

The effects of additional representative cytokines involved in inflammatory responses, such as IFN-γ and TNF-α, have also been investigated. To examine the potential role of IFN-γ, anti-IFN-γ antibody was administered to virus-infected mice[37, 42]. This treatment significantly accelerated the disease course and enhanced Th1 responses towards viral antigens. However, intracerebral injection of recombinant IFN-γ also accelerated the disease similarly[37]. Interestingly, virus-induced demyelination was also accelerated in IFN-γ-receptor deficient mice[13]. These results suggest that IFN-γ is not necessary for developing demyelination, although its presence can exacerbate the disease. Thus, other proinflammatory cytokines, such as TNF-α, may be able to replace this cytokine function. The potential effects of other proinflammatory cytokines on TMEV-induced demyelination have also been investigated. For example, antibodies to TNF-α or IL-12[5, 14–16], as well as administration of rIL-4 (unpublished observation), were able to significantly delay the development and reduce the severity of TMEV-induced demyelination. In addition, treatment of susceptible mice with phosphatidylserine or pentoxifylline, which inhibit the production of TNF-α and IFN-γ, significantly reduced the incidence and severity of disease[14, 54]. These results strongly suggest that the reduction in the proinflammatory cytokine level does provide a beneficial effect on virally induced demyelinating disease. In contrast, PAYA et al.[35] reported that administration of rTNF-α prior to and during viral infection reduced demyelination in the spinal cords. However, these discrepancies may reflect differences in the timing of treatment with respect to viral infection that could be critical for the prevention and/or amelioration of disease[5, 16]. Antibody-mediated reduction in the level of an effector molecule, inducible nitric oxide synthase, acting downstream of these inflammatory cytokines, was also found to be beneficial to the disease[17, 43]. These results strongly suggest that proinflammatory cytokines resulting from the Th1 response to the virus are responsible for the pathogenesis of demyelination, as seen with MS[3, 6, 39].

Potential Pathogenic Mechanisms of Demyelination

The involvement of virus-specific Th1 responses in the pathogenesis of demyelination is supported by strong experimental data. In addition, the fact that autoimmune responses to the major myelin components are followed by the initial inflammatory response to viral antigens supports the potential involvement of "epitope spreading" in the pathogenesis of demyelination[28]. The ability of direct lysis of the host cells by this virus may also contribute to the release of autoantigens that can potentially participate in the induction of the autoimmune responses observed later in the disease. The local

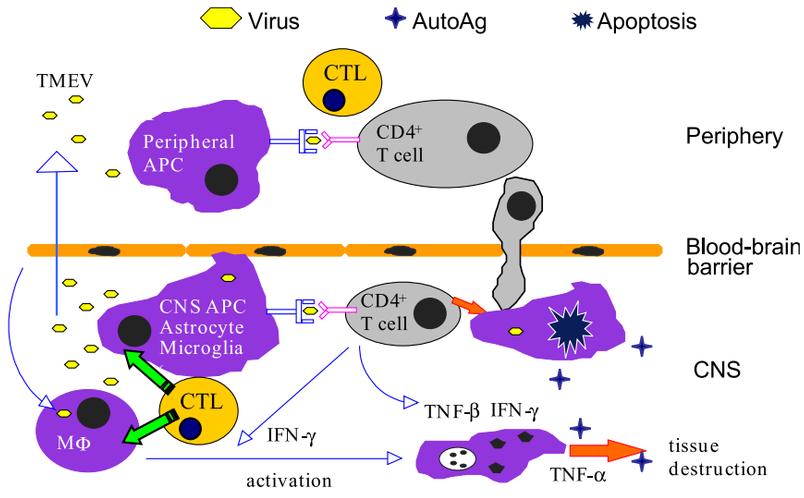

Fig. 2. Potential pathogenic mechanisms involved in the immune-mediated demyelination induced following TMEV infection. Resident glial cells may also be activated by inflammatory cytokines and become competent antigen-presenting cells that further stimulate inflammatory Th1 cell response in the CNS. Such T cell activation may consequently lead to breaching the blood brain barrier by cytokines and/or apoptosis of CNS cells. The tissue destruction leads to further release of autoantigens facilitating autoimmunity. Virus-specific CTLs may be involved in the elimination of virus-infected resident CNS cells and macrophages, limiting pathogenic virus-specific Th1 responses

presentation of viral epitopes to infiltrating $CD4^+$ Th1 lymphocytes in the CNS may also be a critical step for both the initiation and progression of the immune-mediated tissue damage following viral infection (Fig. 2). MHC class II molecules can be expressed on neuroglial cells following exposure to proinflammatory cytokines such as IFN-γ[52] or virus particles[26]. For example, proinflammatory cytokine-activated astrocytes further activate virus-specific $CD4^+$ T cells and, consequently, undergo Fas-mediated apoptosis by the activated T cells *in vivo* as well as *in vitro*[34]. Since astrocytes play an integral role in maintaining the blood-brain barrier[11, 29], such apoptosis of astrocytes most likely contributes to the pathogenesis of TMEV-induced demyelination by compromising the barrier integrity. This is consistent with previous studies suggesting the role of Fas-FasL interaction in MS[10] and, similarly, in autoimmune mouse models of demyelination[25, 44, 49]. In addition, activation of astrocytes induces a variety of immunoregulatory cytokines[21, 51], chemokines attracting various inflammatory cells[47], as well as adhesion and costimulatory molecules[32, 45]. Similarly, virus infection itself may also activate a variety of genes, including chemokines, involved in the immune response (unpublished data). Overall, these may promote the influx of inflammatory cells into the CNS and further amplify immune-mediated demyelination. Inhibition of these critical proinflammatory cytokine functions may be sufficient for the significant delay or amelioration of the disease. Therefore, a precise understanding of the pathogenic mechanisms involved in this virus-induced demyelinating disease will provide important general information regarding the initiation and progression of various tissue-specific autoimmune diseases.

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