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Multiple sclerosis patients have reduced HLA class II-restricted cytotoxic responses specific for both measles and herpes virus

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Summary

It has been previously demonstrated that the generation of measles virus (MV)-specific cytotoxicity (CTL) is reduced in patients with multiple sclerosis (MS). By contrast, CTL specific for influenza virus (FLU) and mumps virus is normal. It is uncertain if reduced CTL is limited to MV in MS patients, or if reduced CTL may be found to other viruses as well. Since MV-specific CTL is predominantly restricted by HLA class II molecules, while FLU-specific and mumps-specific CTL have large HLA class I-restricted components, reduced MV-specific CTL may reflect a broader reduction in HLA class II-restricted CTL in patients with MS. To examine this question we studied the generation of CTL specific for herpes simplex virus type I (HSV). HSV-specific CTL, like MV-specific CTL is predominantly restricted by HLA class II molecules. We found that patients with MS had reduced generation of CTL to both MV and HSV. Most, but not all patients who had reduced generation of CTL to one virus also had a similar impairment with respect to the second virus. Some patients, however, had a reduction in the generation of CTL only to MV or to HSV. These findings extend our earlier observations regarding reduced MV-specific CTL in patients with MS to a second HLA class II-restricted virus, HSV. Such a reduction may reflect discrete impairments in immune function to separate viruses, possibly those that are associated with viral persistence, or may reflect a more generalized defect in HLA class II-restricted CTL.

Introduction

In previous studies, patients with multiple sclerosis (MS) have been shown to have reduced

generation of measles virus (MV)-specific cytotoxicity (CTL) compared to both normal and neurological disease controls (Jacobson et al., 1985). This appears to be the result of a decreased precursor frequency of MV-sensitized effectors rather than suppression of MV-specific CTL by immunoregulatory cells (McFarland et al., 1988). By contrast, CTL responses to the related paramyxovirus mumps virus, and to another RNA based virus, influenza virus, is normal in MS patients (Jacobson et al., 1985; Goodman et al.,

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1989), suggesting that the impaired virus-specific CTL seen in MS patients may be limited to MV. If MV-related immunity is uniquely affected in MS, such an observation may reflect abnormal infection with MV. A similar impairment in the generation of MV-specific CTL has been reported in patients with subacute sclerosing panencephalitis, a disorder known to be the result of a persistent infection with MV (Dhib-Jalbut et al., 1989).

An alternate explanation is that the reduced MV-specific CTL found reflects a more generalized immunological abnormality in MS. Such an abnormality might involve either impaired immunity to multiple viruses or, insofar as MV-specific CTL is predominantly restricted by HLA class II molecules (Jacobson et al., 1987), by a reduction in CTL mediated by CD4⁺ T-cells. Both mumps-specific CTL as well as influenza virus (FLU)-specific CTL have large HLA class I-restricted components (Rotteveel et al., 1988; Goodman et al., 1989). Such HLA class I-restricted CTL are thought to be predominantly mediated by CD8⁺ T-cells.

In this study, we extended our investigation of virus-specific CTL in MS patients to herpes simplex virus type 1 (HSV). HSV, although unrelated to MV, results in viral persistence and CTL directed against HSV, like MV-specific CTL, is thought to be predominantly restricted by HLA class II molecules (Yasukawa and Zarling, 1984a, b).

Materials and methods

Patient and control populations

Sixteen patients with chronic progressive multiple sclerosis, examined in our clinic, and 16 healthy controls, recruited by the NIH Blood Bank, underwent lymphocytapheresis. These studies were reviewed by the Institutional Review Board, and informed consent was obtained prior to any evaluations or procedures. The MS patients were taken from a population of patients receiving placebo in an earlier clinical trial (The Multiple Sclerosis Study Group, 1990). No patient was on immunosuppressive medication at the time of the lymphocytapheresis, or had received such medications within 3 months of the

procedure. Patients had EDSS scores ranging between 3 and 6.5 (Kurtzke, 1965). All MS patients had significant levels of virus-specific antibody to both MV and HSV, indicating prior sensitization to both viruses.

There were no significant differences between MS patients and controls as to either age or sex. The mean age for the control population was $40.5 \pm \text{SD } 14.9$, and for the MS population was $43.6 \pm \text{SD } 9.0$. The female:male ratio for the control group was 0.5, and for the MS group was 0.56.

Lymphocyte preparation

Peripheral blood lymphocytes (PBL) were obtained from patients and normal donors by lymphocytapheresis, while serum was obtained by direct venipuncture. PBL were purified by density gradient centrifugation, and frozen in cryopreservative medium with 10% human serum. They were maintained in a liquid nitrogen vapor freezer until needed.

Virus preparation

MV (Edmonston strain) and HSV (ATCC VR-733) virus pools were prepared by harvesting culture supernatants from virus-infected Vero cells as described elsewhere (Jacobson et al., 1985). Epstein-Barr virus (EBV) pools were prepared by growing an EBV-producing cell line (ATCC B-95-8), and harvesting supernatants.

MV and HSV virus-infected Vero cell monolayers were harvested and sonicated for use as antigen in the enzyme-linked immunosorbent assay (ELISA). The viral titer for MV was determined in a plaque assay, while that for HSV was determined by a tissue culture infective dose assay (TCID) for Vero cells grown in a 96-well plate.

FLU (A/Bangkok/1/79-RX73 (H3N2)) was grown in amniotic fluid, and the viral titer was determined by hemagglutination assay.

Virus-specific cytotoxic cell assay (CTL)

MV-specific and FLU-specific CTL were generated as described previously (Jacobson et al., 1985). Stimulation with MV was performed at a multiplicity of infection (MOI) of 0.1. Stimulation with FLU was performed with a 1:1000 dilution of FLU virus with a titer of 256 hemagglutinating

units/ml (HU/ml). Cells were cultured for 7 days in RPMI 1640 medium with 5% human serum, supplemented with glutamine and antibiotics in a humidified 37°C 5% CO₂ incubator.

HSV-specific CTL were generated in a similar manner; however, stimulation with HSV was performed with 25 μ l of an ultraviolet inactivated preparation of HSV virus with an original titer of 4.75×10^7 by crystal violet staining of HSV-infected Vero cells. Cells were cultured for 10 days in RPMI 1640 medium supplemented with 5% human serum, glutamine and antibiotics as above.

Autologous EBV-transformed B-cell lines were used as targets for all viruses examined. In experiments involving determination of HLA restriction for HSV-specific CTL, HLA-matched and -mismatched EBV-transformed, virus-infected B-cell lines were used. 5×10^6 EBV-transformed B-cells were infected with live virus at the following MOI: MV MOI 0.1; FLU 100 μ l of a virus preparation with a titer of 256 HU/ml; HSV 100 μ l of a virus preparation with a titer of 4.75×10^7 /ml. Virus-infected and uninfected EBV-transformed B-cell lines were then labeled with ⁵¹Cr, and incubated with virus-specific CTL at the following effector to target (E:T) ratios: 40:1, 10:1, 2.5:1. Uninfected autologous EBV-transformed targets were run with each assay as a control for nonspecific activity. Effectors stimulated only with medium, and effectors specific for an irrelevant virus were assayed against each virus-infected target as a control for the integrity of the targets (MV-specific CTL on HSV-infected target and vice versa). Percent lysis was calculated using the following formula:

$$[(\text{Test} - \text{Media}) / (\text{Total} - \text{Media})] \times [100].$$

Statistics

The data was analyzed by Student's *t*-test for independent populations (one-tailed).

Results

Virus specificity of the HSV-CTL assay (Fig. 1)

The specificity of the MV-CTL assay as well as the FLU-CTL assay has been established in pre-

vious investigations. The specificity of HSV-specific CTL was examined by comparison to both MV-specific CTL and FLU-specific CTL. In experiment 1, CTL generated to MV lysed MV-infected targets but failed to lyse HSV-infected targets. Similarly, CTL generated with HSV lysed HSV-infected targets but not targets infected with MV. In experiment 2, CTL generated to FLU lysed FLU-infected targets but not HSV-infected targets, while similar virus specificity was demonstrated by CTL generated to HSV.

HLA restriction pattern of CTL specific for HSV

MV-specific CTL has been shown previously to be predominantly restricted by class II HLA molecules (Jacobson et al., 1984), while FLU-specific CTL is predominantly restricted by class I molecules (Rotteveel et al., 1988). In this investigation, we examined the HLA restriction pattern of the HSV-specific CTL assay by assaying HSV-sensitized effectors on allogeneic B-cell targets that were either matched at HLA class I loci, HLA class II loci, or were HLA mismatched. As shown in Fig. 2, the HSV-sensitized effectors recognized HLA class II-matched targets, but failed to recognize either HSV-infected targets matched at HLA class I, HSV-infected HLA-mis-

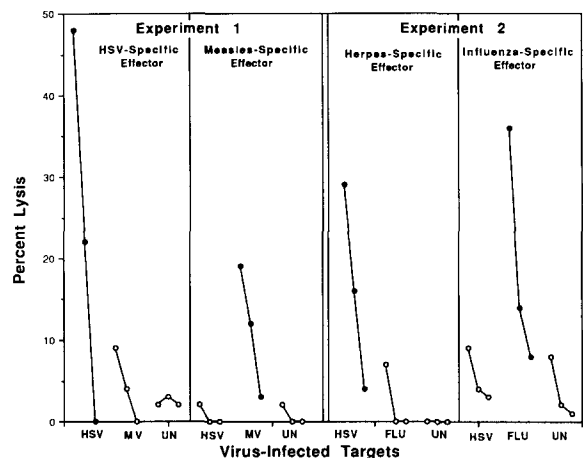


Fig. 1. The virus specificity of the HSV-CTL system is shown. In experiment 1, HSV-sensitized effectors lyse HSV-infected targets, but do not lyse MV-infected or uninfected targets and vice versa. Similarly, in experiment 2, HSV-sensitized effectors lyse HSV-infected targets but do not lyse FLU-infected or uninfected targets. FLU-sensitized effectors lyse FLU-infected targets but not HSV-infected targets.

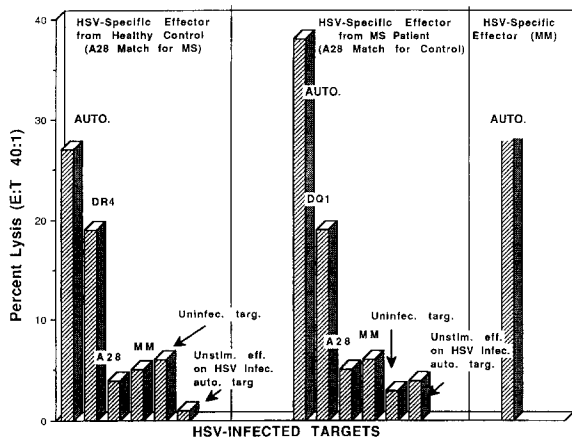


Fig. 2. The HLA restriction of the HSV-CTL system is shown for effectors from both a healthy control as well as a patient with MS. Percent specific lysis is shown at an effector to target ratio (E:T) of 40:1. HSV-sensitized effectors lyse both HSV-infected, autologous targets as well as HSV-infected targets matched at either DR4 and DQ1. By contrast, there is no lysis of HSV-infected targets matched at either A28 or an HLA mismatched (MM) target. These targets are, however, readily lysed by their autologous HSV-sensitized effectors.

matched targets, or uninfected targets. By contrast, the class I-matched and -mismatched targets were readily killed by their autologous effectors. Similar results in HLA restriction were obtained for CTL from MS patients as were found for controls.

In a subsequent experiment examining the HLA restriction of HSV-specific CTL (see Fig. 3), none of the four HLA-mismatched targets, nor the four HLA class I-matched targets evidenced lysis above that seen for the uninfected autologous control. By contrast, both HLA class II-matched targets as well as the autologous HSV-infected target (positive control) were readily lysed by HSV-sensitized effector. This indicates that HSV-CTL, like MV-CTL is predominantly restricted by HLA class II molecules, and is supportive of observations by other investigators regarding the HLA class II restriction of HSV-specific CTL (Yasukawa and Zarling, 1984a, b).

MV and HSV-specific CTL in MS patients and controls

MV and HSV-specific CTL was examined in both MS patients as well as controls. Percent lysis

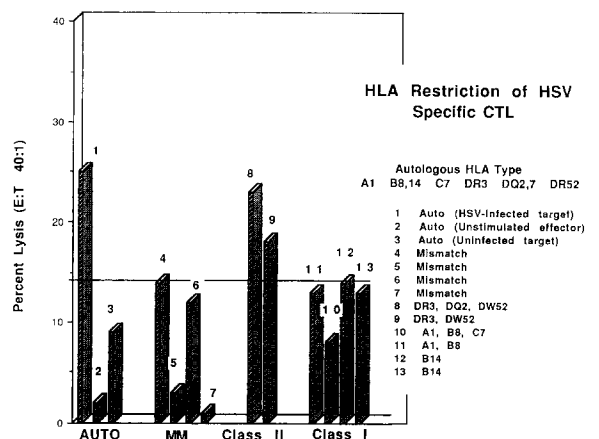


Fig. 3. The HLA restriction pattern for HSV-sensitized effectors taken from a healthy control is shown at an effector to target ratio (E:T) of 40:1. HSV-specific CTL is shown both on the autologous HSV-infected target, HSV-infected targets matched at HLA class I molecules, HSV-infected targets matched at HLA class II molecules, and HSV-infected HLA-mismatched targets. A horizontal line has been drawn at the top of the panel of HLA-mismatched targets. The HSV-infected autologous target is readily lysed by the HSV-sensitized effector, as are both HLA class II-matched targets. By contrast, none of the HLA class I-matched targets are lysed at levels greater than that found for the panel of HLA-mismatched targets. This suggests that HSV-sensitized CTL is predominantly restricted by HLA class II molecules.

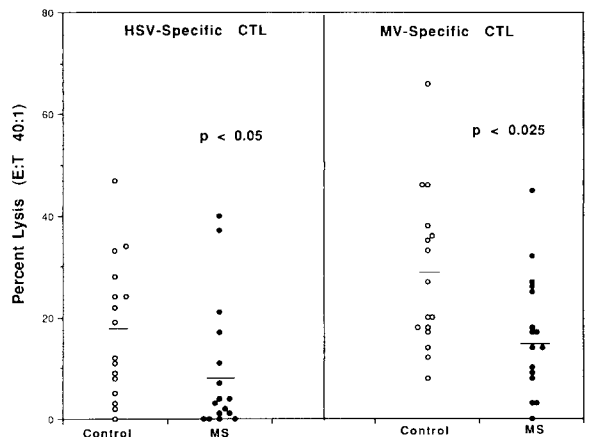


Fig. 4. MV-specific and HSV-specific CTL are shown for both healthy controls and patients with MS. Percent lysis at an effector to target (E:T) ratio of 40:1 is shown for virus-sensitized effectors assayed on autologous virus-infected targets. Both HSV-specific, as well as MV-specific CTL are reduced in MS.

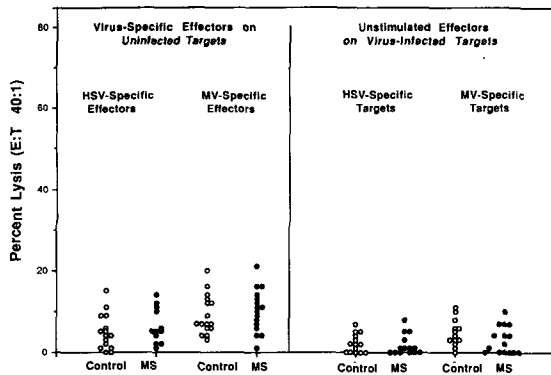


Fig. 5. Nonspecific lysis is shown for both healthy controls and patients with MS at an effector to target (E:T) ratio of 40:1. There is no difference between MS patients and controls in nonspecific lysis either for virus-specific effectors assayed on uninfected targets or for unstimulated effectors assayed on virus-infected targets.

at an E:T ratio of 40:1 is shown in Fig. 4. The control population had a mean MV-specific CTL value of 28.4% with an SD of 15.56%. For the MS population, the mean MV-specific CTL was 16.8% \pm SD 11.9% ($p < 0.025$). For HSV, the control population had a mean HSV-specific CTL of 17.6% \pm SD 13.5%, while the MS population had a mean HSV-specific CTL of 9.4% \pm SD 12.9% ($p < 0.05$). By contrast, there was no difference between controls and MS patients in percent lysis of virus-infected targets by PBL stimulated only with medium (Fig. 5) or in percent lysis of virus-stimulated targets assayed upon uninfected autologous targets.

Correlation between HSV-specific CTL and MV-specific CTL in patients with multiple sclerosis

We examined the relationship between MV-specific CTL and HSV-specific CTL in MS patients and controls to see if there was any correlation between reduced MV-specific CTL and reduced HSV-specific CTL in MS, supportive of a generalized reduction in HLA class II-restricted CTL (see Table 1). None of the healthy controls had reduced generation of CTL specific for both viruses. Four patients with MS (25%) had unequivocal reduction in both MV-specific and HSV-specific CTL, and an additional four patients (50% total) had some reduction in CTL specific for both HSV and MV. This reduction in

TABLE 1

THE RELATIONSHIP BETWEEN HSV-SPECIFIC AND MV-SPECIFIC CTL IN MS PATIENTS AND CONTROLS (PERCENT LYSIS E:T = 40:1)

No.	Controls		No.	MS patients	
	HSV	MV		HSV	MV
1	33	33	1	1	32
2	24	20	2	37	10
3	3	27	3 ^a	11	14
4	11	35	4	17	45
5	8	36	5 ^b	4	0
6	47	14	6 ^b	4	3
7	34	17	7	21	8
8	0	38	8 ^a	7	17
9	5	66	9	1	26
10	19	18	10 ^b	0	9
11	2	20	11	2	27
12	12	18	12	40	18
13	28	12	13	3	25
14	22	8	14 ^a	0	14
15	24	46	15 ^a	3	17
16	9	46	16 ^b	0	3

^a Partial reduction in CTL specific for both HSV and MV.

^b Unequivocal reduction in CTL specific for both HSV and MV.

CTL generation was not the result of a generalized impairment in PBL function in these patients insofar as FLU-specific CTL was normal in all but one of the eight patients with reduced MV and HSV-specific CTL (see Table 2). There was, however, no correlation between HSV-specific CTL and MV-specific CTL in either the MS or control populations.

TABLE 2

INFLUENZA VIRUS (FLU)-SPECIFIC CTL IN MS PATIENTS WITH REDUCED CTL TO BOTH MV AND HSV (PERCENT LYSIS E:T = 40:1)

Patient No.	FLU-CTL
3	35
5	23
6	39
8	7
10	35
14	29
15	99
16	30

Discussion

In previous studies, patients with MS have been found to have reduced generation of MV-specific CTL when compared to both healthy and neurological disease controls (Jacobson et al., 1985). By contrast, the generation of CTL specific for both mumps virus and influenza virus is normal in MS (Jacobson et al., 1985; Goodman et al., 1989). In this investigation we found that the generation of CTL specific for HSV is also reduced in MS. Thus, the CTL defect previously thought limited to MV can be extended to at least one other virus. There are at least two explanations for these findings: MS patients may have a generalized defect in CD4⁺ T-cell function or, alternately, MS patients may have impaired cellular immunity to a number of viruses, including but not necessarily limited to MV and HSV.

The idea that reduced generation of CTL specific for MV and HSV may reflect a generalized impairment in CD4⁺ T-cell function stems from the observation that both MV as well as HSV induce an immune response that appears to be predominantly restricted by HLA class II-restricted CTL. Such CTL are thought to be primarily mediated by CD4⁺ T-cells. Reduced CD4⁺ T-cell-mediated CTL in MS is intriguing insofar as MS patients have been repeatedly shown to generate elevated amounts of antibody to multiple viruses (including both MV and HSV) (Norrby et al., 1974a, b; Baig et al., 1989). The generation of such antibody is also dependent upon CD4⁺ T-cells (Van Binnendijk et al., 1989). Similar elevations of antibody production with reduced CTL have been reported in a number of animal models of autoimmune disease, including murine graft-versus-host disease, and murine lupus (Gleichmann et al., 1984; Theofilopoulos et al., 1985; Moser et al., 1987; Via et al., 1987, 1990; Via and Shearer, 1988). In addition, reduced MV-specific CTL has been reported in patients with HAM/TSP, a demyelinating disorder caused by the HTLV-I virus that is similar to MS (Jacobson et al., 1989). Further, patients with early, asymptomatic infection with HIV-1, the virus responsible for the acquired immunodeficiency syndrome (AIDS), also have elevated anti-

body production and reduced CTL (Shearer et al., 1986; Eales et al., 1988). This is intriguing insofar as it is during early HIV-1 infection that AIDS patients are most susceptible to autoimmune disorders such as inflammatory demyelinating polyneuropathy. Thus, it is possible that reduced HLA class II-restricted CTL may predispose patients to the development of autoimmune disease.

How might a reduction of virus-specific HLA class II-restricted CTL lead to autoimmune disease? It has been suggested that HLA class II-restricted CTL may play a role in regulating the immune response to virus by the lysis of antigen-presenting cells that display viral antigens (Otenhoff and Mutis, 1990; Strober and James, 1990). Such lysis would tend to limit the immune response, and may inhibit the generation of self-reactive clones. A primary immunoregulatory abnormality in MS, whether genetic or acquired, that results in reduced HLA class II-restricted CTL may permit an exaggerated immune response upon stimulation with common viruses. Such exaggerated immunity may lead to the expansion of clones cross-reactive with myelin proteins, with resultant demyelinating disease.

The possibility that a generalized abnormality in HLA class II-restricted CTL might be present in MS patients was previously examined in relation to mumps virus by other members of our laboratory. In that investigation, no difference was found in mumps-specific CTL assayed upon HLA class II-restricted targets between MS patients and controls (Goodman et al., 1988). However, the generation of HLA class II-restricted CTL in CTL responses marked by both CD4⁺ and CD8⁺ effectors (as is the response to mumps virus) may differ from that generated in virus-specific CTL responses that are predominantly HLA class II restricted. CD8⁺ T-cells have been reported to provide help, and may increase the generation of CD4⁺ CTL in systems where both CD8⁺ and CD4⁺ CTL precursors are generated. In this regard, it is instructive that in both animal models of autoimmunity and in early HIV infection, the CTL defect was correctable with interleukin-2 (IL-2) (Via and Shearer, 1990).

In our study, we examined the relationship between MV-specific CTL and HSV-specific CTL

in our patient and control groups. Although many MS patients had reduced CTL specific for both HSV and MV in our study, we found no significant correlation between MS patients with reduced MV-specific CTL and those with reduced HSV-specific CTL. It is possible that low CTL, when found in our assay system, may reflect gross reduction in virus-specific CTL, and that the assay may not be adequate to identify more subtle differences. In addition, the overall level of virus-specific CTL generated during primary infection is thought to be affected by numerous factors such as general health and age at the time of infection. Thus, a defect in HLA class II-restricted CTL could escape detection in our small sample, given the presence of other variables influencing CTL generation.

An alternate explanation for the reduced MV and HSV-specific CTL found in MS is that MS patients may have abnormal infection with neurotropic viruses such as MV and HSV. Such abnormal infection may reflect various factors including genetic background, viral strain, and age at the time of initial exposure to virus. Abnormal infection with virus might then initiate MS either by resulting in viral persistence, mimicry of neural antigens, or in disordered immune regulation.

MV is known to result in a persistent infection, subacute sclerosing panencephalitis (SSPE). In this disorder, patients have elevated titers of MV-specific antibody but reduced MV-specific CTL, similar to that found in MS (Dhib-Jalbut et al., 1989). It is postulated that reduced MV-specific CTL in SSPE may contribute to viral persistence in affected patients. However, while genetic material hybridizing to MV-specific probes has been found in both MS as well as control brain (Hasse et al., 1984), unlike SSPE, repeated attempts to isolate live virus from MS brain have failed, weighing against a hypothesis of viral persistence in MS. Similarly, while some examples of molecular mimicry exist (Fujinami and Oldstone, 1985), this seems an unlikely explanation for the induction of MS by either MV or HSV.

That viruses may initiate autoimmune disease is clear from animal models of demyelinating disease caused by virus. Infection with both MV

as well as coronavirus in Lewis rats has resulted in the generation of T-cells that proliferate in response to myelin basic protein (MBP) (Watanabe et al., 1983; Liebert et al., 1988). Following expansion in vitro, such MBP-specific T-cells can transfer experimental allergic encephalitis. A similar anti-MBP response has been reported in humans following MV encephalitis (Johnson et al., 1984). It is possible that if individuals have reduced cellular immunity to virus, they may be more susceptible to central nervous system (CNS) involvement upon infection with common viruses. Such CNS involvement may lead to sensitization to myelin antigens in MS patients, with or without viral persistence. Repeat exposure to, or activation of, the virus may then result in the induction of a delayed-type hypersensitivity response directed against myelin antigens, with subsequent demyelinating disease.

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