Contrasting effects of immunosuppression on herpes simplex virus type I (HSV I) induced central nervous system (CNS) demyelination in mice

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Summary

We previously reported that lip inoculation of Herpes simplex virus type I (HSV I) in specific strains of mice would induce multifocal brain demyelination (MBD). The mechanisms mediating the development of MBD are unknown. In this study, five inbred strains of mice (C57BL/6J, Balb/cByJ, A/J, SJL/J, PL/J) immunosuppressed with either irradiation (IR), cyclophosphamide (CY), or cyclosporin A (CP) along with three immune deficient strains (C57BL/6J nu/nu, Balb/cByJ nu/nu, C57BL/6J bg/bg) were lip inoculated with HSV I to determine the effect of immunosuppression on viral spread throughout the brain and the development of demyelination during the acute stage of infection. Mortality increased in all groups when compared with controls but was greatest in A/J, SJL/J, and PL/J strains, where all mice died before day 6 PI. In contrast with immunocompetent C57BL/6J mice where virus is restricted to the brainstem, virus spread throughout the brain of immunosuppressed C57BL/6J, C57BL/6J nu/nu, and C57BL/6J bg/bg mice. Despite viral spread throughout the brain of immunosuppressed C57BL/6J, C57BL/6J nu/nu, Balb/cByJ and Balb/cByJ nu/nu mice, MBD did not develop. MBD did develop however, in both HSV I infected C57BL/6J bg/bg and CP treated Balb/cByJ mice. Immunosuppression of HSV I infected Balb/cByJ mice prevents the development of demyelination at the trigeminal root entry zone (TREZ) of the brainstem while in Balb/cByJ nu/nu mice, the extent of demyelination at TREZ was reduced and delayed when compared with immunocompetent controls. These results suggest that the immune system plays an important role in limiting viral spread in the brain as well as in the development of demyelination at TREZ and of MBD throughout the brain during the acute phase of infection. Virus alone does not induce MBD in this animal model of virus induced CNS demyelination but is a prerequisite for its development.

Introduction

Experimental animals infected with herpes simplex virus type I (HSV I) develop many features of herpetic infection observed in man including: acute disease (Kastrukoff et al., 1982), latent infection of the peripheral nervous system (PNS) (Cook and Stevens 1973), and viral nucleic acid sequences in the central nervous system (CNS) (Cabrera et al. 1980; Rock and Fraser 1983). Of interest is the ability of HSV I to induce CNS demyelination in mice following peripheral inoculation with virus. The lesions, which are characterized by demyelination, relative preservation of axons, and a mononuclear cell (MNC) infiltrate, were originally identified in Balb/c mice and thought to be restricted to the trigeminal root entry zone (TREZ) of the brainstem (Kristensson et al. 1979; Townsend 1981). Recent studies however, indicate that other murine strains (A/J, SJL/J, and PL/J) develop multifocal brain demyelination (MBD) following lip inoculation with HSV I (Kastrukoff et al. 1987. Kastrukoff et al. 1992). All three murine strains develop acute stage MBD. This

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phase is characterized by the sequential appearance of lesions (TREZ > brainstem > cerebellum > cerebral hemispheres) beginning 6 days post-infection (PI) and ending by day 24 PI (Kastrukoff et al. 1992). In addition, A/J and PL/J mice develop chronic stage MBD where new lesions appear sporadically throughout the brain for up to 8 weeks PI in the former and beyond 28 weeks PI in the latter strain (Kastrukoff et al. 1992).

Although the mechanisms mediating the development of HSV I induced brain demyelination are not well understood, the sequential spread of virus throughout the brain during the first 12 days PI appears to be a prerequisite for the development of MBD in A/J, SJL/J, and PL/J mice (Kastrukoff et al. 1987; Kastrukoff et al. 1992). In C57BL/6J mice, virus is limited to the TREZ and MBD does not develop (Kastrukoff et al. 1987). The presence of virus throughout the brain is not the only factor determining the development of MBD. In Balb/cByJ mice, virus spreads sequentially throughout the brain, with viral titers equal to those found in A/J mice, but demyelination is restricted to the TREZ (Kastrukoff et al. 1987). The presence of MNCs in the demyelinating lesions suggest that the immune system is also an important factor (Kastrukoff et al. 1989).

This study examines the role of the immune system in limiting viral spread throughout the brain of mice lip inoculated with HSV I and in the development of brain demyelination during the acute phase. HSV I infected immune deficient (nude and beige mice) and immunosuppressed (total body irradiation, cyclophosphamide, cyclosporin A) C57BL/6J mice are used to study the role of the immune system in limiting viral spread. HSV I infected immunosuppressed A/J, SJL/J, and PL/J mice are used to study the role of the immune system in the development of acute phase MBD. HSV I infected immune deficient (nude mice) and immunosuppressed Balb/cByJ mice are used to determine the role of the immune system in the development of acute phase demyelination.

Materials and methods

Animals

Inbred A/J, Balb/cByJ, C57BL/6J, PL/J, SJL/J, Balb/cByJ (nu/nu), C57BL/6J (nu/nu), ·and C57BL/6J (bg/bg) male mice were obtained from Jackson Laboratories, Bar Harbor, ME. All strains were maintained for 14 days prior to use at 10–12 weeks of age.

Virus and cells

HSV I, laboratory strain 2, was used throughout these studies. Virus, propagated on BHK-21 cells and plaque-assayed on CV-1 cells, was lip inoculated as previously described, with all mice receiving the same

inoculum (Kastrukoff et al. 1982). The titer was 1.0×10^7 PFU (plaque forming units) per ml.

Viral titrations

Groups of 5 mice from each strain (C57BL/6J, Balb/cByJ, A/J, PL/J, SJL/J) immunosuppressed with either total body irradiation (IR) or cyclophosphamide (CY) were killed on day 4 PI. Brain homogenates were obtained by freeze-thawing of tissue 3 times followed by grinding in Ten Broeck glass tissue grinders. Serial dilutions of tissue homogenates were plaque-assayed (Kastrukoff et al. 1982).

Immunosuppression

Irradiation. Groups of 20 mice of each strain (C57BL/6J, Balb/cByJ, A/J, SJL/J, PL/J) were irradiated (IR) with 8 Gy of total body gamma irradiation delivered by a cobalt source one day prior to lip inoculation with HSV I. IR non-infected mice and non-IR HSV I infected mice of each strain served as controls.

Cyclophosphamide. Groups of 20 mice of each of the 5 inbred murine strains received 200 mg/kg of cyclophosphamide (CY) IP 2 days before and 3 days following lip inoculation with HSV. CY treated non-infected mice and non-CY treated HSV I infected mice of each strain served as controls.

Cyclosporin A. Cyclosporin A (CP) was administered i.p. to groups of 20 mice of each of the 5 inbred strains for 2 days prior and 3 days following lip inoculation with HSV I. Drug was administered at 50 mg/kg. CP treated non-infected mice and non-CP treated HSV I infected mice of each strain served as controls.

Immunodeficient mice

Groups of 20 mice from each strain (C57BL/6J (nu/nu), C57BL/6J (bg/bg), Balb/cByJ (nu/nu)) were lip inoculated with HSV I. Non-infected mice from each strain served as controls.

Serum anti-HSV antibodies

Serum was assayed by solid-phase radioimmunoassay (RIA) for the presence of anti-HSV antibodies as previously described (Kastrukoff et al. 1982). Plates had been prepared by infection of wells confluent with CV_1 cells with HSV I at a MOI (multiplicity of infection) of 1.0. Following a 2-h incubation, 50 μl 0.15% glutaraldehyde in 0.15 M PBS, pH 7, was added to each well and allowed to incubate for 5 min. Twenty-five μl 0.3 M glycine-HAS solution (455 mg Na H_2PO_4 · H_2O, 1.8 g Na_2HPO_4 · 7 H_2O 8.2 g NaCl, 800 mg NaN_3, distilled H_2O to 1 l), pH 6.8, was then added and held for a further 5 min. The cells were then washed repeatedly with HAS + 1% agamma-globulin horse serum. Serum (25 μl of a 1:2000 dilution) was added in triplicate to wells of a 96-well microtiter plate and removed after 1.5 h. The plates were washed 3

TABLE 1

SURVIVAL, VIRAL TITERS, IMMUNOHISTOCHEMISTRY, AND HISTOPATHOLOGY IN IMMUNOCOMPROMISED MICE INFECTED WITH HSV I

	Survival rates		Viral titers in the brain (PFU/GM) (mean±SD)	Immunohistochemistry (viral AG)						Histopathology					
				Day 6 PI		Day 9 PI		Day 12 PI		Day 6 PI		Day 9 PI		Day 12 PI	
	Day 6 PI	Day 12 PI	Day 4 PI	TREZ	CB	TREZ	CB	TREZ	CB	TREZ	CB	TREZ	CB	TREZ	CB
I. Immunocompetant															
C57BL/6J+HSV I	20/20	20/20	NR	+	–	+	–	+	–	MNC	NP	MNC	NP	MNC	NP
Balb/cByJ+HSV I	20/20	18/20	NR	+	–	+	+	+	+	DEM	NP	DEM	NP	DEM	NP
II. Immunosuppression															
A. Irradiation (8GY)															
C57BL/6J	20/20	20/20	–	–	–	–	–	–	–	NP	NP	NP	NP	NP	NP
C57BL/6J +HSV I	20/20	16/20	$(5.00 \pm 0.38) \times 10^5$	+	–	+	+	+	+	HI	NP	HI	HI	HI	HI
Balb/cByJ	20/20	19/20	–	–	–	–	–	–	–	NP	NP	NP	NP	NP	NP
Balb/cByJ +HSV I	18/20	10/20	$(7.50 \pm 0.42) \times 10^6$	+	–	+	+	+	+	HI	NP	HI	HI	HI	HI
A/J	20/20	18/20	–	–	–	–	–	–	–	NP	NP	NP	NP	NP	NP
A/J+HSV I	0/20	0/20	$(2.80 \pm 0.25) \times 10^8$	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
SJL/J	20/20	19/20	–	–	–	–	–	–	–	NP	NP	NP	NP	NP	NP
SJL/J+HSV I	0/20	0/20	$(2.90 \pm 0.29) \times 10^8$	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
PL/J	20/20	18/20	–	–	–	–	–	–	–	NP	NP	NP	NP	NP	NP
PL/J+HSV I	0/20	0/20	$(3.10 \pm 0.16) \times 10^8$	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
B. Cyclophosphamide															
C57BL/6J	20/20	20/20	–	–	–	–	–	–	–	NP	NP	NP	NP	NP	NP
C57BL/6J+HSV I	20/20	19/20	$(3.50 \pm 0.16) \times 10^5$	+	–	+	+	+	+	HI	HI	HI	HI	HI	HI
Balb/cByJ	20/20	20/20	–	–	–	–	–	–	–	NP	NP	NP	NP	NP	NP

Balb/cByJ													
+HSV I	19/20	$(5.54 \pm 0.65) \times 10^{6}$	+	–	+	+	+	HI	HI	HI	HI	HI	HI
A/J	20/20	–	–	–	–	–	–	NP	NP	NP	NP	NP	NP
A/J+HSV I	0/20	$(1.12 \pm 0.18) \times 10^{8}$	NR	NR	NR	NR	NR	NP	NP	NP	NR	NR	NR
SJL/J	20/20	–	–	–	–	–	–	NP	NP	NP	NP	NP	NP
SJL/J+HSV I	0/20	$(1.38 \pm 0.36) \times 10^{8}$	NR	NR	NR	NR	NR	NP	NR	NR	NR	NR	NR
PL/J	20/20	–	–	–	–	–	–	NP	NP	NP	NP	NP	NP
PL/J+HSV I	0/20	$(1.42 \pm 0.76) \times 10^{8}$	NR	NR	NR	NR	NR	NP	NR	NR	NR	NR	NR
III. Immune deficient													
C57BL/6J (nu/nu) +HSV I	18/20	NR	+	–	+	+	+	NP	NP	NP	NP	MNC	NP
Balb/cByJ (nu/nu) +HSV I	17/20	NR	+	–	+	+	+	NP	NP	NP	NP	DEM	NP
C57BL/6J (bg/bg) +HSV I	11/20	NR	+	–	+	+	+	DEM	DEM	DEM	MBD	DEM	MBD
IV. Cyclosporin A													
C57BL/6J	20/20	NR	–	–	–	–	–	NP	NP	NP	NP	NP	NP
C57BL/6J+HSV I	20/20	NR	+	+	+	+	+	MNC	MNC	MNC	NP	MNC	NP
Balb/cByJ	20/20	NR	–	–	–	–	–	NP	NP	NP	NP	NP	NP
Balb/cByJ+HSV I	15/20	NR	+	+	+	+	+	DEM	DEM	DEM	MBD	DEM	MBD
A/J	19/20	NR	–	–	–	–	–	NP	NP	NP	NP	NP	NP
A/J+HSV I	0/20	NR	NR	NR	NR	NR	NR	NP	NP	NP	NR	NP	NP
SJL/J	19/20	NR	–	–	–	–	–	NP	NP	NP	NP	NP	NP
SJL/J+HSV I	0/20	NR	NR	NR	NR	NR	NR	NP	NR	NR	NR	NR	NR
PL/J	16/20	NR	–	–	–	–	–	NP	NP	NP	NP	NP	NP
PL/J+HSV I	0/20	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

NR = no results, NP = no pathology; MNC = mononuclear cell infiltrate; DEM = demyelinating lesions; HI = hemorrhagic infiltrates; MBD = multifocal brain demyelination.

times and rabbit anti-mouse antisera specific for IgM or IgG were added and incubated for 1.5 h. After washing, radio-iodinated goat anti-rabbit antibodies (32000 cpm per 25 μl) were added and incubated for 1.5 h. After washing, the level of bound radioactivity to the cells was determined in a gamma-counter. Results are expressed as cpm − naive serum. Levels of sensitivity were calculated to be 0.1 ng for both IgM and IgG.

Histopathology

Methods used in the preparation, sectioning, and staining of murine brain tissue have previously been described (Kastrukoff et al. 1987). Briefly, animals were perfused in vivo with 80% methanol, 10% acetic acid, and 10% formaldehyde (FAM). CNS tissue was dehydrated in alcohol and toluol, embedded in paraffin, and sectioned serially. Six-μm thick coronal sections were made through the cerebral hemispheres and brain stem–cerebellum. Sections were stained with either hematoxyllin-eosin (H&E), Luxol fast blue/cresyl fast violet (LFB-CFV), or Luxol fast blue/Holmes silver nitrate (LFB-HSN). Because of the large number of sections that were generated in these studies, brain stem–cerebellum sections were studied in detail. All sections were coded and examined in a blinded fashion.

Immunohistochemistry

The unlabeled antibody enzyme method of immunohistochemistry of Sternberger was employed (Sternberger et al., 1970). Briefly, mice were perfused in vivo with FAM. CNS tissue was dehydrated through ethanol and embedded in paraffin. Six-μm thick sections were obtained and then treated with 0.5% H_2O_2 in methanol for 30 min. The sections were then rehydrated, washed in TBSG (Tris-saline, 0.05 M, pH 7.6, plus 1% normal goat serum) and rabbit polyclonal anti-HSV I antiserum (1:500) (DAKO) was applied for 12 h at 4°C. Tissue was then washed in TBSG and goat anti-rabbit IgG anti-serum (1:250) was applied at room temperature for 30 min (Cappell Laboratories). The rewashed sections were then treated with rabbit PAP (Cappell Laboratories) (1:25) at room temperature for 30 min, washed and stained with 0.03% 3,3'-diaminobenzydine (Sigma) plus 0.05% H_2O_2 in Tris-saline (0.05 M, pH 7.6). Washed sections were then treated with 0.03% OsO_4, counterstained, and examined.

Electron microscopy

Mice were perfused in-vivo with 1.5% paraformaldehyde and 2% gluteraldehyde in PBS. CNS tissue was fixed in 3.1% gluteraldehyde at 4°C for 24 h, sectioned on a vibrotome, post-fixed, stained with 1% OsO_4 for 60 min and dehydrated in alcohol. Sections were embedded in Epon, sectioned and examined with a Phillips 300 electron microscope (Kastrukoff et al. 1989).

Results

Mortality and viral titers in the brain

HSV I infection of immunosuppressed or immunodeficient mice of all strains results in greater mortality (Table 1) when compared with virus infected immunocompetent controls (Kastrukoff et al., 1987, 1992).

A/J, SJL/J, and PL/J mice are particularly vulnerable with all HSV I infected animals immunosuppressed with either IR, CY, or CP dying within 6 days of lip inoculation.

Viral titers in the brain on day 4 PI following immunosuppression with IR varies with the murine strain (Table 1). In C57BL/6J mice, brain titers are 5.0×10^5 PFU/g while in Balb/cByJ mice, titers are 7.5×10^6 PFU/g. In contrast, titers in A/J, SJL/J, and PL/J mice are greater then 10^8 PFU/g. Similar results are obtained in mice immunosuppressed with CY. In C57BL/6J mice, the titer is 3.5×10^5 PFU/g while in Balb/cByJ the titer is 5.5×10^6 PFU/g. Viral titers in A/J, SJL/J, and PL/J mice all exceed 10^8 PFU/g of brain tissue.

Effect of immunosuppressive agents on anti-HSV antibody

Depression of serum anti-HSV antibody was used as an index of the efficacy of immunosuppression with either IR or CY during the acute stage of infection. As previous studies indicated that anti-HSV neutralizing antibodies can not be readily detected during the first 12 days PI in virus infected mice (Knoblich et al. 1983) but anti-HSV IgM and IgG can be identified using either RIA or ELISA techniques (Kastrukoff et al. 1982; Knoblich et al. 1983), a solid phase RIA was employed to identify the presence of anti-HSV antibody.

Results are given in Table 2. Both IR and CY are effective in reducing anti-HSV IgM and IgG production in both virus infected C57BL/6J and Balb/cByJ mice.

TABLE 2

SERUM ANTI-HSV ANTIBODIES IN IMMUNOSUPPRESSED MICE LIP INOCULATED WITH HSV I

	Sample No. [a]	IgM (day 12 pi) [b]	IgG (day 12 pi) [b]
C57BL/6J	20	125 ± 25	2603 ± 113
C57Bl/6J (irradiated, 8 Gy)	16	0	35 ± 23
C57BL/6J (cyclo 200 mg/kg)	19	13 ± 8	60 ± 20
Balb/cByJ	19	160 ± 30	2157 ± 154
Balb/cByJ (irradiated, 8 Gy)	10	0	22 ± 6
Balb/cByJ (cyclo 200 mg/kg)	17	24 ± 11	49 ± 10

cyclo = cyclophosphamide.
[a] Pooled serum samples examined from each group of mice.
[b] The serum results are expressed as cpm per 25 μl of a 1:2000 dilution (mean ± SD).

Viral spread and brain pathology in HSV-I infected immunosuppressed mice

In HSV-I infected immunocompetent C57BL/6J mice, viral antigen is restricted to the trigeminal root entry zone (TREZ). MNCs are present at TREZ but brain demyelination does not develop. In contrast, viral antigen is present throughout the brain of HSV I infected IR C57BL/6J mice including the TREZ by day 6 PI (Fig. 1A) and the cerebellum (CB) by day 9 PI (Fig. 1B) (Table 1). Neither demyelination nor MNC infiltrates are observed in the brains of these mice but rather hemorrhagic infiltrates including those at TREZ by day 6 PI (Fig. 1C) and those in the CB by day 9 PI (Fig. 1D) (Table 1). Hemorrhagic infiltrates are not observed in IR controls. Similar results are observed in HSV I infected C57BL/6J mice immunosuppressed with CY instead of IR.

In HSV I infected immunocompetent Balb/cByJ mice, viral antigen is present throughout the brain while demyelination is restricted to the TREZ. In HSV I infected IR Balb/cByJ mice, viral antigen is again present throughout the brain but demyelination does not develop (Table 1). Rather hemorrhagic infiltrates are observed throughout the brain. Hemorrhagic infiltrates are not observed in IR controls. Similar results are observed in HSV I infected Balb/cByJ mice immunosuppressed with CY but MBD does not develop.

Viral spread and brain pathology in HSV I infected immunodeficient mice

Viral antigen is present throughout the brain of HSV I infected C57BL/6J (nu/nu) mice including the TREZ by day 6 PI and the CB by day 9 PI (Table 1). Multifocal brain demyelination does not develop in these mice. MNC infiltrates are observed at the TREZ but their appearance is delayed until day 12 PI (Table 1).

Viral antigen is also present throughout the brain of HSV I infected Balb/cByJ (nu/nu) mice including the TREZ by day 6 PI and the CB by day 9 PI (Table 1). Multifocal brain demyelination does not develop in these mice. MNC infiltrates and demyelination are observed at the TREZ but their appearance is delayed until day 12 PI (Fig. 2A and B) compared with day 6 PI in immunocompetent controls (Table 1).

In HSV I infected C57BL/6J (bg/bg) mice, viral
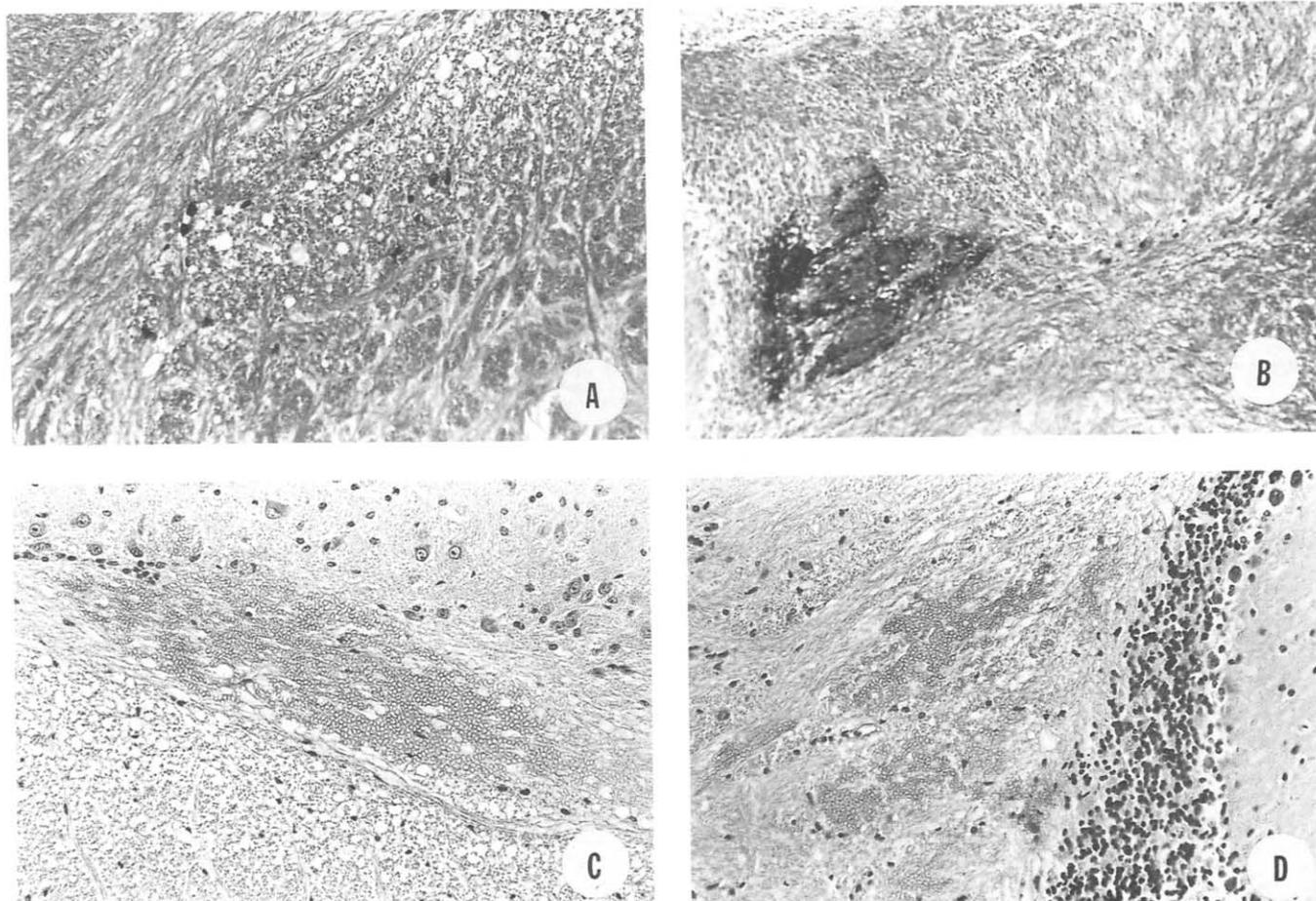
Fig. 1. HSV I infected irradiated (8Gy) C57BL/6J male mouse. (A) Viral antigen in the TREZ region. Anti-HSV polyclonal anti-sera plus PAP stain, 6 days PI ×100. (B) Viral antigen in the cerebellum. Anti-HSV polyclonal anti-sera plus PAP stain, 9 days PI, ×100. (C) Hemorrhagic infiltrate at the TREZ, 6 days PI, H&E stain, ×200. (D) Hemorrhagic infiltrate in the cerebellum, 9 days PI, H&E stain, ×200.

antigen is also present throughout the brain, appearing at the TREZ by day 6 PI and at the CB by day 9 PI (Table 1). MNC infiltrates and demyelination appear at the TREZ of these mice by day 6 PI (Fig. 3A). Multifocal brain demyelination also develops in these mice (Fig. 3B and C). The lesions are characterized by demyelination, relative preservation of axons, and a MNC infiltrate (Fig. 3D).

Viral spread and brain pathology in HSV I infected mice treated with cyclosporin A

In HSV I infected C57BL/6J mice treated with CP (50 mg/kg), viral antigen is restricted to the TREZ. A MNC infiltrate is present at the TREZ by day 6 PI but MBD does not develop (Table 1).

In HSV I infected Balb/cByJ mice treated with CP, viral antigen is present throughout the brain including the TREZ by day 6 PI and in the CB by day 9 PI (Table 1). Demyelination at the TREZ appears by day 6 PI. Unlike the control group, CP treated HSV I infected Balb/cByJ mice do develop multifocal CNS

Fig. 2. TREZ lesions developing in Balb/cByJ nu/nu mice are characterized by the delayed appearance of a MNC infiltrate (day 12 PI) and limited demyelination. (A) Demyelinating lesion at TREZ, 12 days PI, LFB-CFV stain, ×100. (B) Higher power photomicrograph of lesion in (A), LFB-CFV stain, ×200.

demyelination (Fig. 4A). Lesions developing in the CB by day 9 PI are characterized by demyelination and a MNC infiltrate (Fig. 4B and C) along with relative preservation of axons (Fig. 4D). The characteristics of these lesions are confirmed by EM studies (Fig. 4E).

Discussion

We previously presented results which indicated that HSV I can induce multifocal brain demyelination (MBD) in specific strains of mice following lip inoculation with virus (Kastrukoff et al. 1986, 1987). MBD develops in two stages. In the acute stage, MBD develops sequentially throughout the brain while in the chronic stage it develops sporadically (Kastrukoff et al. 1992). The mechanisms mediating the development of MBD remain unknown. The results of this study, using immunocompromised mice suggest: that the immune system plays a role in limiting viral spread in the brain following peripheral inoculation with virus, that the effectiveness of the immune system in limiting viral spread in the brain depends on the murine strain, and that other mechanisms may play a role in limiting viral production in the brain. The results suggest that the immune system plays a role in the development of demyelination at the trigeminal root entry zone (TREZ) and MBD developing during the acute stage. Also, viral spread throughout the brain is a requirement for the development of demyelination.

HSV I infection of the immunocompromised murine strains results in greater mortality when compared with immunocompetent controls, suggesting that the immune system plays an important role in the survival of these animals. Furthermore, the higher viral titers in the brains of immunocompromised mice compared with immunocompetent controls (Kastrukoff et al. 1987) suggests that the immune system plays a role in clearing virus from the brain. Differences in both mortality and viral titers in the brain do occur among the different immunocompromised murine strains. One possible explanation for these differences is that C57BL/6J and Balb/cByJ mice were only partially immunosuppressed. This is unlikely as the methods employed in the protocol were designed to induce complete immunosuppression (Worthington et al. 1980) and this is reflected in the low levels of anti-HSV antibody in all strains. It is more likely that other mechanisms besides the immune system play a role. Differences in resistance to HSV I have been identified in primary glial cultures derived from different murine strains (Kastrukoff et al., 1987; Thomas et al. 1991) and may play a role in limiting viral production and spread in the brain.

In immunocompetent C57BL/6J mice, virus spread is limited to the brainstem (Kastrukoff et al. 1987). In this study, immunosuppression of C57BL/6J mice with
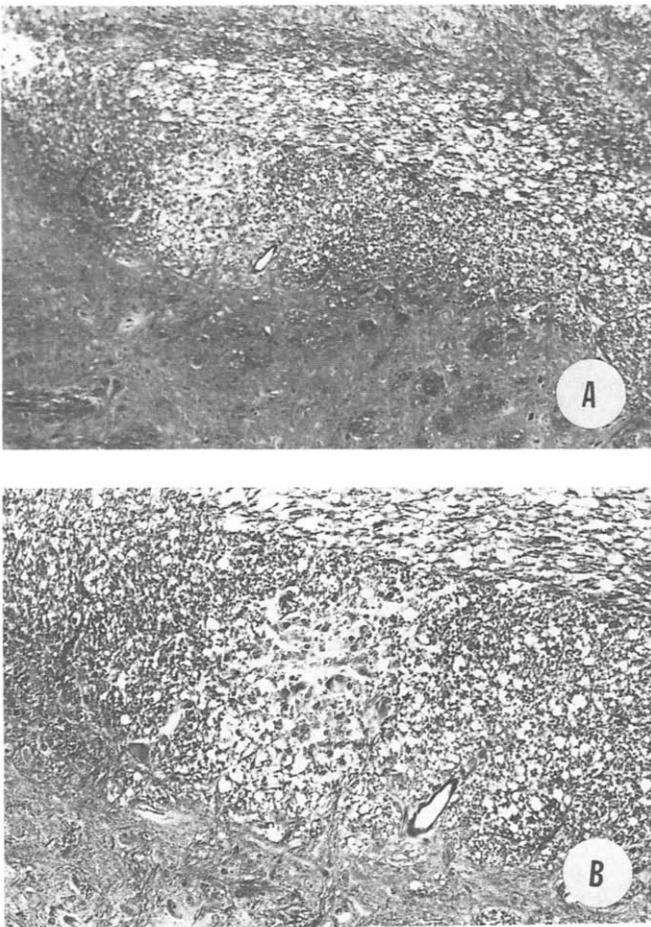
either IR or CY results in the spread of virus throughout the brain. This would support a role for the immune system in limiting viral spread in the CNS. Virus also spreads throughout the brain in immune deficient C57BL/6J nu/nu mice and in C57BL/6J bg/bg mice. The former strain lacks normal T-lymphocytes while the latter strain lacks normal NK cell activity (Roder and Duwe 1979; Morahan et al. 1982). The results suggest that both T-cells and NK cells play a role in limiting the spread of virus in the CNS. T-cells have previously been implicated as playing an important role in limiting viral spread in the CNS (Kapoor et al. 1982) but a similar role for NK cells has only been identified following intraperitoneal inoculation of virus (Habu et al. 1984). Additional studies are required to confirm the importance of NK cells as other immune abnormalities do occur in bg/bg mice, to determine which subsets of T-cells play a role, and to determine the site of action of the immune cells in limiting the spread of virus in the CNS. In immunosuppressed (IR or CY) Balb/cByJ mice, virus is present throughout the brain similar to HSV I infected immunocompetent controls.

Mortality rates are higher in the former group however, as are viral titers in the brains when compared with immunocompetent controls (Kastrukoff et al. 1987).

Results of this study also suggest that MBD developing during the acute stage is immune mediated but requires, as a prerequisite, the spread of virus throughout the brain. The presence of virus in the CNS is insufficient to produce these lesions. In both C57BL/6J and Balb/cByJ mice immunosuppressed with either IR or CY, virus is present throughout the brain but demyelinating lesions do not develop. In these mice the pathology is characterized by hemorrhagic infiltrates throughout the brain. Similar results are obtained in immune deficient mice (C57BL/6J nu/nu and Balb/cByJ nu/nu mice) where virus is present throughout the brain but MBD is not observed. In contrast, MBD does develop in C57BL/6J bg/bg mice following the spread of virus throughout the brain. When these results are taken together, they suggest that T-cells play an important role in the development of MBD. Presumably, in bg/bg mice, abnormal NK
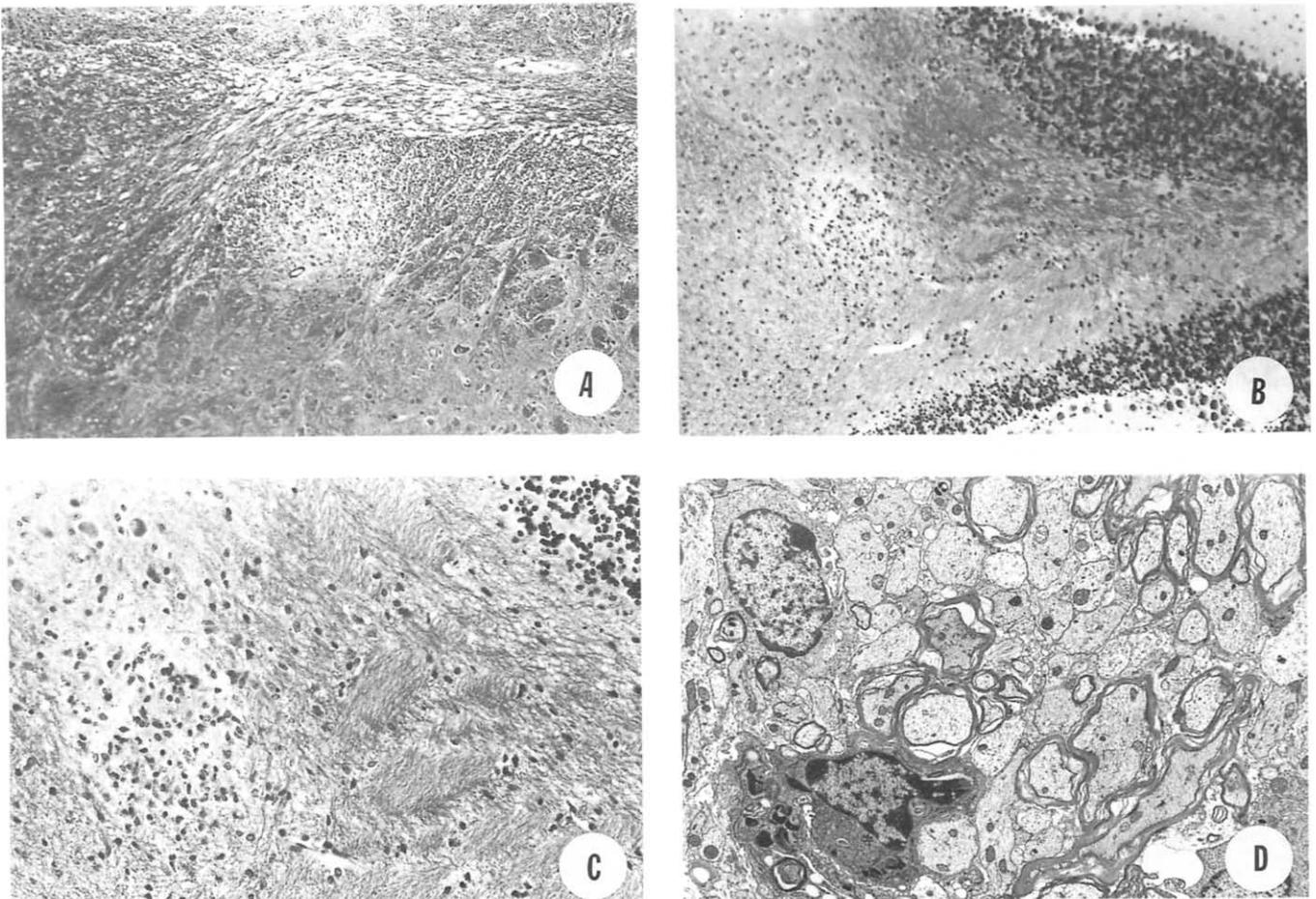
Fig. 3. Multifocal CNS demyelination developing in C57BL/6J (bg/bg) mice following lip inoculation with HSV I. (A) Demyelinating lesion at TREZ, 6 days PI, LFB-CFV stain, ×100. (B) Cerebellar demyelinating lesion, 9 days PI, LFB-CFV, ×100. (C) High power photomicrograph of demyelinating lesion in (B), LFB-CFV, ×200. (D) EM photomicrograph of a cerebellar lesion. The lesions are characterized by demyelination, relative preservation of axons, and MNC infiltrates, ×1350.

cell function allows the spread of virus in the CNS while T-cells are involved in the development of the demyelinating lesions. In nu/nu mice, abnormal T-cells are unable to limit the spread of virus in the CNS and participate in the development of these lesions. This interpretation is supported by similar results derived from a murine model of HSV I induced keratitis. T-cells were identified as playing an important role in the development of pathology in that model (Metcalf and Kaufman 1976, Metcalf et al. 1979; Russell et al. 1984).

MBD also develops in Balb/cByJ mice treated with low dose CP. Here again MBD develops only after the spread of virus throughout the brain. In contrast, C57BL/6J mice does not develop MBD after CP, but virus does not spread throughout the brain. These results indicate that under certain circumstances, Balb/cByJ mice can develop MBD. The mechanisms involved in the production of demyelination in this case are not clear. As an immunosuppressant, CP has many actions (Hess et al. 1982) including inhibition of helper T-cell function (Kahan 1989), inhibition of selected
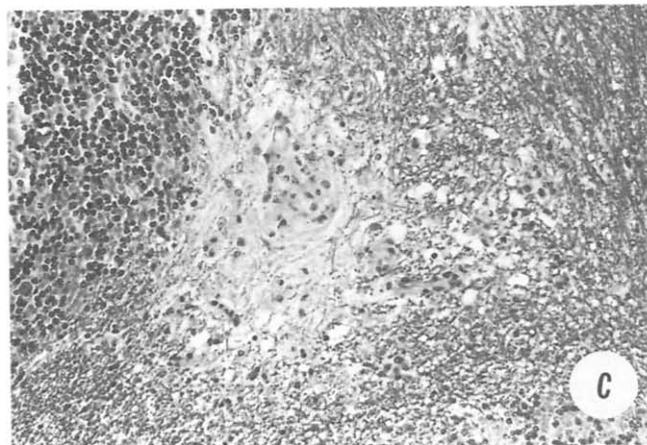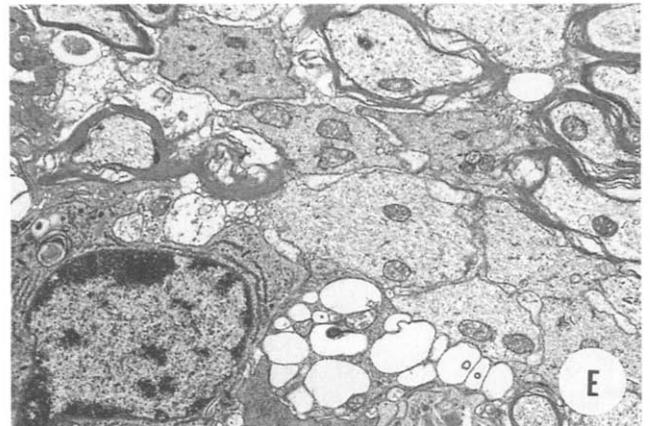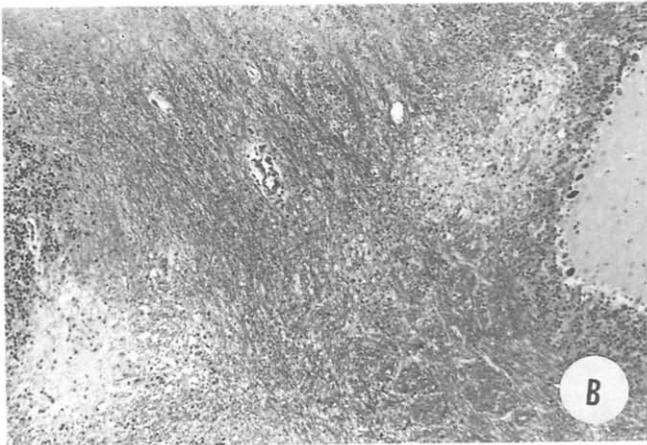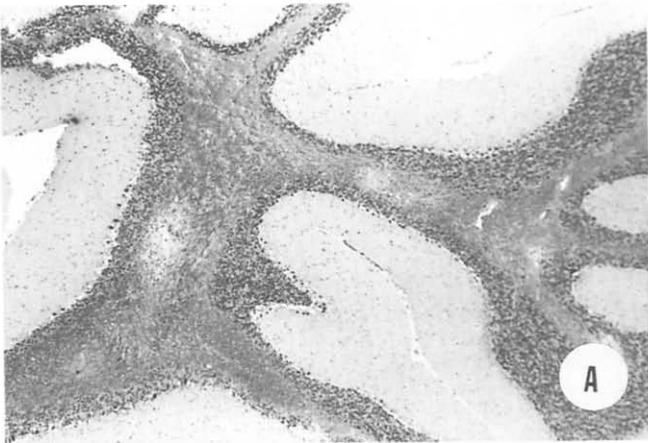
Fig. 4. Multifocal CNS demyelination developing in Balb/cByJ mice following lip inoculation with HSV I and treatment with cyclosporin A (50 mg/kg) (A) Multiple lesions in the white matter of the cerebellum, 9 days PI, LFB-CFV, ×40. (B) Higher power photomicrograph of demyelinating lesions in (A), LFB-CFV, ×100. (C) Mononuclear cells are present in the demyelinating lesion, LFB-CFV, ×200. (D) Preservation of axons in a demyelinating lesion in the cerebellum, LFB-Holmes stain, ×400. (E) EM photomicrograph of cerebellar lesion. Characteristics include demyelination, relative preservation of axons, and a MNC infiltrate, ×1600.

cytotoxic T-cell function (Orosz et al. 1988), and sparing of suppresser T-cell function. None of these actions easily explain why MBD develops in CP treated Balb/cByJ mice. CP also has a number of non-immunosuppressant actions including the ability to inhibit direct cell to cell transmission of HSV I (McKenzie et al. 1987). One might speculate that in Balb/cByJ mice, CP inhibits the transneuronal spread of HSV I known to occur in the CNS (Ugolini et al. 1989; Margolis et al. 1989) and result in increased viral escape at the synapses. Virus, contained by surrounding glial cells similar to that seen with pseudorabies infection of the CNS (L.W. Enquist, personal communication), could become the target of the immune system resulting in demyelination.

Results of this study also suggest that the immune system plays a role in the development of demyelination at the TREZ of the brainstem. Immunocompetent Balb/cByJ mice develop demyelination at the TREZ following lip inoculation with HSV I (Kastrukoff et al. 1987). In this study, immunosuppression of Balb/cByJ mice with either IR or CY prevents the development of demyelination at TREZ despite spread of virus throughout the brain. These results suggest that demyelination at TREZ is immune mediated and are consistent with reported of other investigators (Townsend and Baringer 1979; Townsend 1981, 1985). In Balb/cByJ nu/nu mice, the development of demyelination at the TREZ is delayed and less extensive compared with immunocompetent controls. These results suggest the T-cells contribute to the development of demyelination at TREZ but are not the only cell type involved. This is consistent with results from both EM (Kastrukoff et al. 1989) and immunohistologic studies (Chan et al. 1989) which suggest that both macrophages and T-cells participate in the development of these lesions. Large numbers of macrophages along with limited numbers of both CD4+ and CD8+ T-cells have been identified in close contact with HSV infected cells in areas of myelin loss.

A murine model of HSV I induced CNS demyelination is only one of a number of different experimental models of virus induced demyelination which have implications for human demyelinating disease. These models have recently been reviewed in detail (Dal Canto 1990; Rice and Kastrukoff 1993). Although specific details of the pathology and pathogenesis vary from one model system to another, similarities do exist. These include: the ability of a number of different viruses and specific viral strains to induce CNS demyelination, susceptibility of specific strains of animals to develop demyelination, an acute stage of infection followed by the development of chronic demyelination, a persistent or latent viral infection, and in some cases a role for the immune system in the development of demyelination. Unlike HSV I, the initial demyelinating

lesions observed with canine distemper virus are not necessarily associated with inflammatory infiltrates. This observation suggested that different mechanisms were operating during the acute and persistent stages of infection. Further observations suggested that the demyelination developing during the acute stage was dependent on astrocytic infection. In contrast, the development of the persistent stage of infection appeared to depend on the effectiveness of antibody-mediated clearance of virus during the acute stage of infection. Similarly, the initial demyelinating lesions seen with coronaviruses (e.g. JHM strain of murine hepatitis virus) preceded the development of an inflammatory infiltrate. This observation along with failure of immuno-suppression to prevent demyelination (Weiner 1973) suggested that demyelination developing during the acute stage was produced by direct viral cytopathic effect on the myelinating cells. In contrast, the development of demyelination during the chronic stage appears to result from an interplay between persistent virus and the immune system. Visna virus can also induce acute and chronic stage CNS demyelination but unlike canine distemper virus and JHM virus, the demyelinating lesions developing during the acute stage appear to be immune mediated as immunosuppression with CY and anti-thymocyte serum prevents their development (Nathanson et al. 1976). It is unclear if the demyelinating lesions developing during the chronic stage are also immune mediated. Similarly in Theiler's murine ecephalomyelitis virus induced demyelination, the acute stage of demyelination appears to be immune mediated. Immunosuppression with CY and anti-thymocyte serum prevents the development of inflammation and demyelination (Lipton and Dal Canto 1976). The development of demyelination during the chronic stage appears to result from an interplay between persistent virus and the immune system.

Studies with HSV I induced CNS demyelination along with other models of virus induced demyelination may help define novel mechanisms by which common human viruses may induce demyelination and has implications for human demyelinating diseases such as MS.

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