CORONAVIRUS SD-INDUCED IMMUNOREGULATORY DISTURBANCES IN A MURINE MODEL OF DEMYELINATION

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INTRODUCTION

We previously reported the isolation of coronaviruses while working with autopsy tissue from patients with multiple sclerosis[1] and have been investigating the pathogenesis of these viruses in mice. Inoculation of three to four week old C57BL/6 mice with coronavirus SD induces a syndrome characterized by hindlimb anesthesia, paresis, or both between 4 and 9 days post-infection.[2] We now report the finding of an unusual immunologic derangement in these mice.

Detection of Infectious Virus and Viral Antigen in Various Tissues

Infectious virus was recoverable in high titer from the brain (3.7×10^4 pfu) and spinal cord (1.4×10^4 pfu), only rarely and in very low titer from the spleen and liver for the first four days post-infection (Figure 1). Virus could not be isolated at any time from the blood, heart, lung, thymus, kidney, or duodenum (data not shown). Infectious virus was not detected in the liver by day 5, the spleen by day 6, and the brain and spinal cord by day 7 (Figure 1). Virus was detectable by co-cultivation of minced brain tissue on 3T3 (17CL-1) cells as late as day 12 post-infection, after which all methods of isolation (tissue homogenate, co-cultivation, and cell fusion) proved unsuccessful. An extensive search for viral particles by electron microscopy revealed only an occasional viral particle in necrotic cell debris of the temporal cortex on days 4 through 6 post-infection.

Despite the relatively brief time when infectious virus was recoverable from various tissues, viral antigen persisted for a long period of time (Figure 2).

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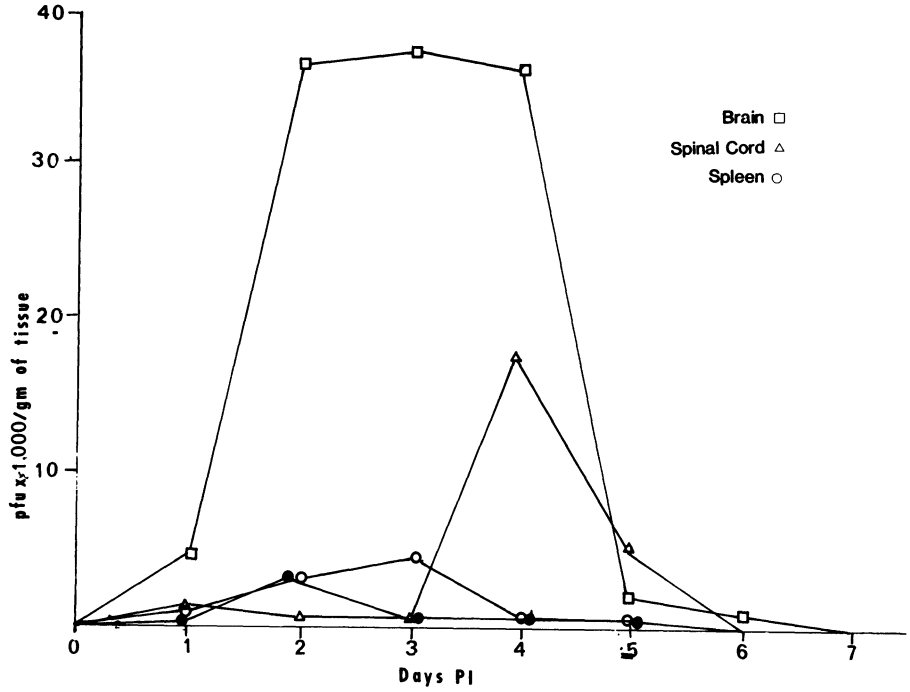

Fig. 1. Infectious virus detected in 10% homogenates of brain (squares), spinal cord (triangles), and spleen (circles) by plaque assay on delayed brain tumor cells after intracerebral inoculation of SD virus in weanling C57BL/6 mice. PI indicates post-inoculation; pfu, plaque forming units.

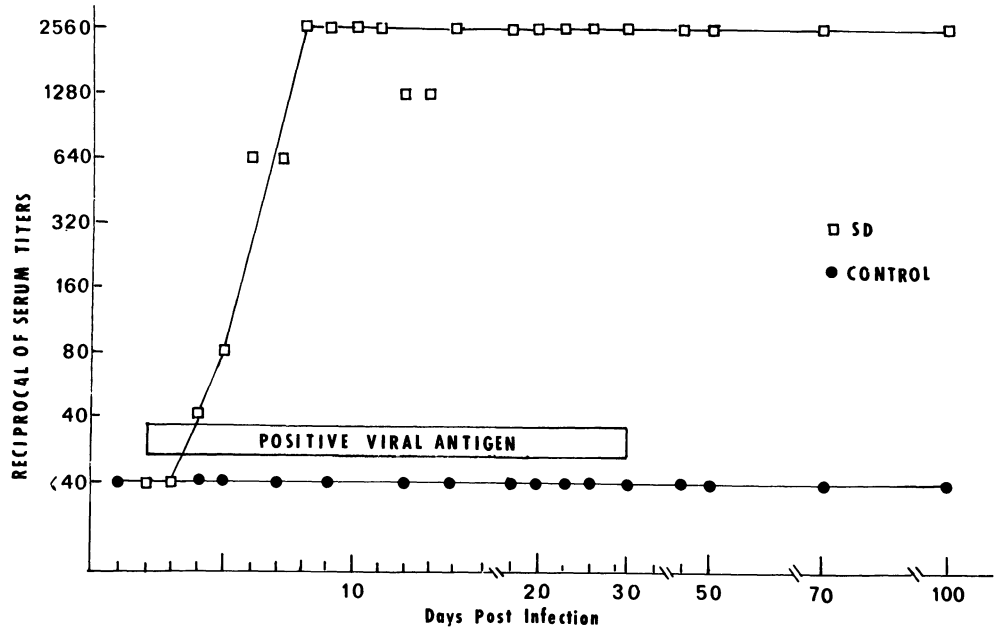

Fig. 2. Serum antibody titers are given as the reciprocal of the serum dilution which gave 50% reduction in the number of plaques using SD virus. Viral antigen was detected by autoradiography with antisera to coronavirus SD out to day 30 PI and then intermittently to day 473 PI.

Immunoregulatory Disturbances

To determine whether disturbances in immune function accompany infection, the proliferative response of spleen cells from mice infected 11 days earlier with coronavirus to the T cell mitogen concanavalin (Con A) and B cell mitogen lipopolysaccharide (LPS) was assessed and compared to that of untreated mice and from mice injected with heat-inactivated virus. Spleen cells from mice injected with live virus were found to proliferate poorly in response to both Con A (Figure 3) and LPS relative to those from untreated mice or from mice given heat-inactivated virus (Figure 4). The inability to proliferate in response to these mitogens was evident even 42 days post-infection (Figure 5), a time at which infectious virus cannot be recovered from tissues (Figure 1).

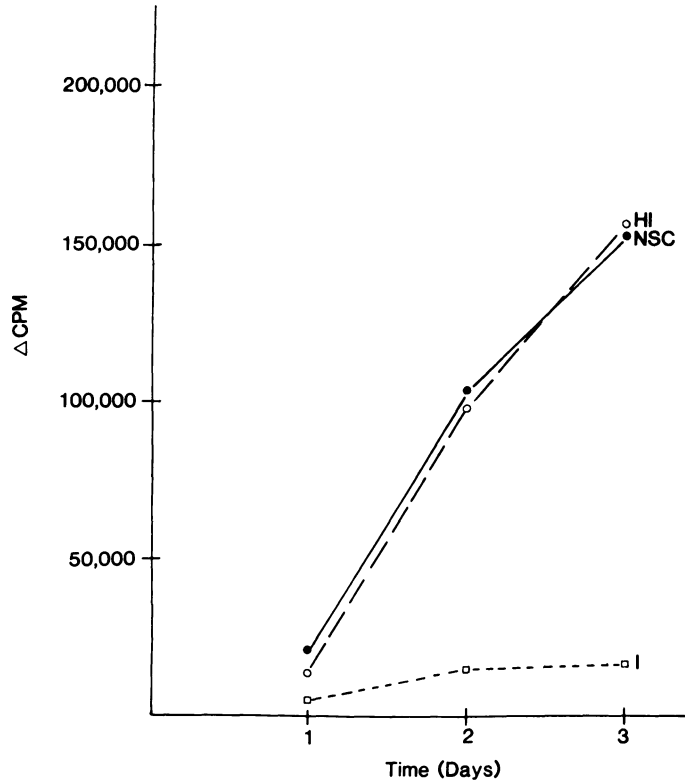

Fig. 3. Spleen cells (1×10^5) from untreated (NSC), mice given heat-inactivated (HI) or live (I) virus 11 days earlier were optimally stimulated with Con A (0.5 ug) and DNA synthesis assessed daily for 3 days using tritiated thymidine incorporation (^{3}HTdR). CPM, counts per minute.

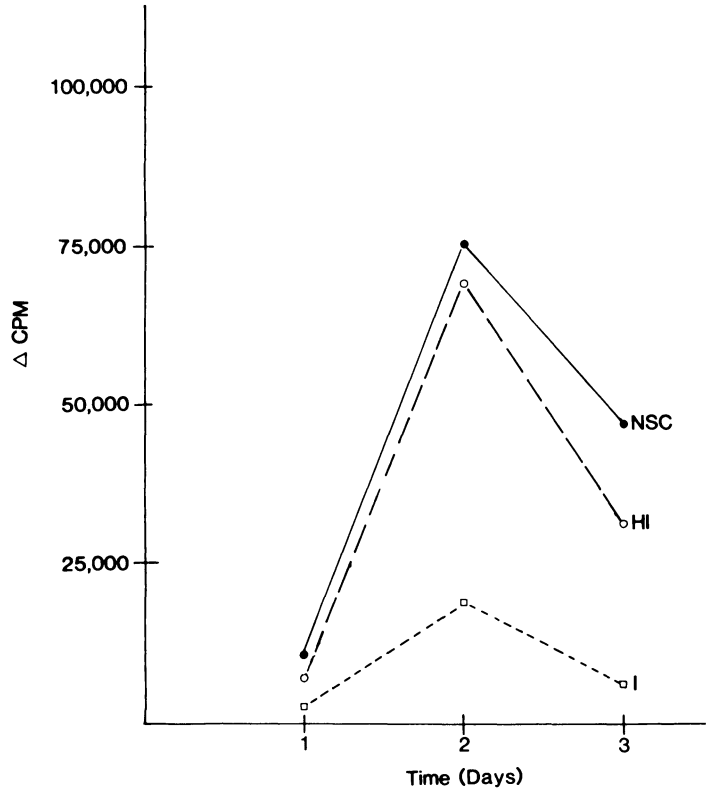

Fig. 4. Spleen cells (1 x 10^5) from untreated (NSC), mice given heat-inactivated (HI) or live (I) virus 11 days earlier were optimally stimulated with LPS (10 ug) and DNA synthesis assessed daily for 3 days using tritiated thymidine incorporation (^{3}HTdR).

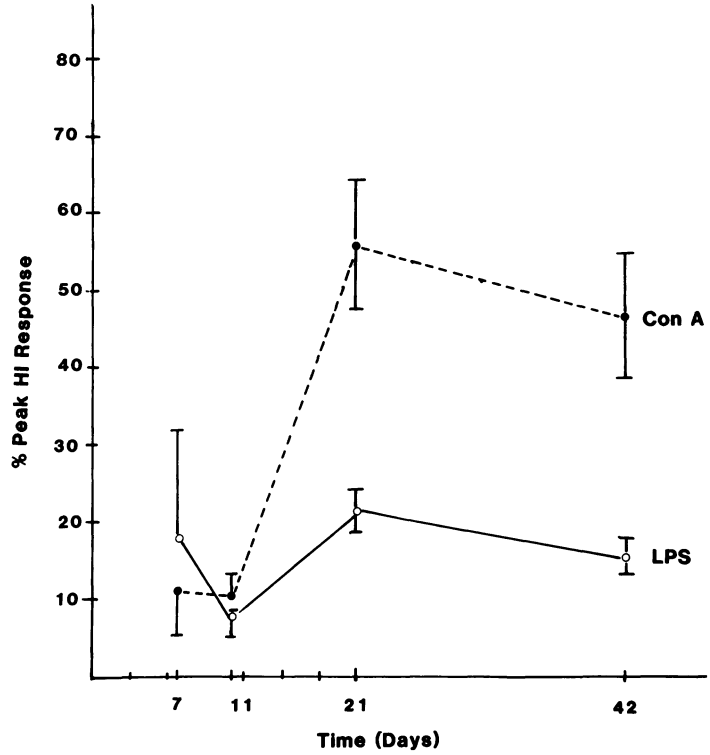

Fig. 5. Spleen cells (1×10^5) from mice were cultured at the indicated times following virus administration and stimulated with Con A (0.5 ug) or LPS (10 ug) and DNA synthesis assessed at the indicated times using tritiated thymidine incorporation (^{3}HTdR).

An examination of the cellular basis for this defect revealed no differences between virus-infected and control groups with respect to total spleen cell numbers or to percentages of T and B (Lytl+ and Ig+ respectively) cells (Table 1). Interleukin 2 (IL2) production (Table 2) and IL2 receptor expression after stimulation with Con A (Table 3) were normal as was Con A-induced immune interferon synthesis (data not shown).

DISCUSSION

One current hypothesis about the etiology of multiple sclerosis in humans is that it is a viral-induced autoimmune disorder confined to the central nervous system. Many viruses have been suggested as having a role in multiple sclerosis, including coronaviruses.[1] Although the human data at this time has not supported the above hypothesis, recent animal studies have shown a link between primary coronavirus-induced demyelinating encephalomyelitis and secondary development of an autoimmune demyelinating disease, experimental autoimmune encephalomyelitis (EAE).[3] The profound immunoregulatory disturbances seen in the present study are not seen either in multiple sclerosis in humans or EAE in animals; however, a similar "immunodepression" has been described in other coronavirus virus-induced experimental disorders.[4,5]

Table 1. Splenic Size and Cell Composition

	Total Cell Count/Spleen	Percent Positive Staining	
	($x10^7$)	Ig	Lyt 1
Untreated	5.23	31.3	45.3
Heat Inactivated	5.90	34.6	58.6
Live Virus	5.26	25.0	63.6

Spleen cells from untreated mice or mice given heat-inactivated or live virus 11 days earlier were counted for total number of lymphoid cells and stained with an optimal concentration of fluorescein-conjugated anti-mouse IgM or anti-mouse Lyt1 and then analyzed on the fluorescence-activated cell sorter (FACS).

Table 2. IL2 Production

	CPM
Untreated	34,000
Heat Inactivated	30,000
Live Virus	26,000
Rat Factor	32,000

Spleen cells from untreated mice or mice given heat-inactivated or live virus 11 days earlier were stimulated with Con A and the supernatants were assessed for IL2 activity up to 7 days of culture on the IL2 dependent CTLL line. Shown are the peak proliferative responses of CTLL cells at a 1:10 dilution of the sample calculated by linear regression.

Table 3. IL2 Receptor Expression

	Percent Positive Staining
Untreated	68.7
Heat Inactivated	64.5
Live Virus	81.2

Spleen cells from untreated mice or mice given heat-inactivated or live virus 11 days earlier were stimulated with Con A for 36 hours. These cells were stained with a monoclonal antibody to the murine IL2 receptor (7D4), and then analyzed on the FACS.

The immunologic derangement observed in the present study may be related to disease pathogenesis or may merely reflect the response of the immune system to a viral infection, or a combination of both. It is unlikely, however, that infectious virus is causing these immunoregulatory disturbances, since none could be isolated at a time when these disturbances were present. However, the persistence of viral antigen with resultant persistent immunostimulation may cause the immunodepression. This effect may be mediated by persistent elevated levels of circulating interferon, an observation previously noted in MHV-3 induced immunodepression.[4] The underlying cause of this unusual immunologic derangement and its relation to latent infection is under investigation.

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