Spontaneous Production of Interleukin-2 and Interleukin-3 by Spleen Cells from Mice Infected with Mouse Hepatitis Virus Type 4

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**Spleen cells from mice, 4 to 60 days after infection with mouse hepatitis virus type 4, produced interleukin-2,
as well as interleukin-3, in the absence of exogenous stimulants in vitro. This unique lymphokine production
by mouse hepatitis virus type 4 infection was controlled by host genes.**

During our studies on mitogen-induced lymphokine production by murine lymphoid cells, we noted that some spleen cells from conventionally housed mice, which were at risk of infection with mouse hepatitis virus (MHV), showed a moderate lymphokine production without exposure to mitogens, which may cause some problems in studying the biological significance of lymphokines. The purpose of this study was to ascertain whether spleen cells from experimentally MHV-infected mice produce lymphokines in vitro in the absence of exogenous mitogens.

Female 6- to 8-week-old BALB/cCr (BALB/c) mice were used. The mouse breeding colonies had been routinely checked serologically for the absence of MHV, Sendai virus, and *Mycoplasma pulmonis* (4). The JHM strain of MHV (MHV-4) was propagated on DBT cells and a plaque assay was performed as described previously (7).

Culture supernatants of spleen cells from either infected or uninfected mice were prepared as follows. BALB/c mice were inoculated intraperitoneally with 10^4 PFU of MHV-4. A single-cell suspension was prepared by using chilled RPMI 1640, supplemented with 10% heat-inactivated fetal bovine serum, 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 5×10^{-5} M 2-mercaptoethanol, and antibiotics. A 1-ml quantity of a suspension containing 5×10^6 cells was distributed into each well of 12 multiwell plates and incubated at 37°C for 48 h, which was the optimal condition for spontaneous lymphokine production by the cells. After centrifugation, supernatants were collected, exposed to a UV lamp, and filtered through a 0.22-μm-pore-size membrane filter. Interleukin-2 (IL-2) and interleukin-3 (IL-3) activities of the supernatants were quantitated in bioassays by using IL-2-dependent CTLL-2 and IL-3-dependent FDC-P2 cell lines, respectively, as described previously (8, 10). The results for IL-2 are expressed in international units per milliliter; for IL-3, the results are expressed

as stimulation index (counts per minute of cells incubated with supernatants [final dilution, 1:4]/counts per minute of cells incubated with assay medium alone).

Although neither IL-2 nor IL-3 activity was detected in the supernatants of spleen cell cultures from uninfected mice, those from mice at 4 days postinfection (p.i.) or later showed IL-2, as well as IL-3, activities (Fig. 1A and 2). Both activities peaked at 0.5 or 1 month p.i. However, spleen cells from mice 2 months p.i. barely produced these lymphokines. The time courses of IL-2 production by the spleen cells and virus titer in the spleen were not parallel (Fig. 1). There was a positive correlation between IL-2 and IL-3 activities of the supernatants ($r = 0.851$) (Fig. 2).

Spleen cells from seven inbred strains of mice, either uninfected or 1 month after infection with MHV-4, were examined for spontaneous lymphokine production in vitro by the same procedure (Table 1). The culture supernatants from MHV-infected STS/A mice showed extremely high IL-2 activities, whereas those from infected A/J mice failed to produce IL-2. The results indicated that spontaneous lymphokine production by spleen cells from MHV-infected mice was apparently dependent on the host genotype. However, the strain distribution pattern of the response was not identical to that of susceptibility to the virus (9, 15). Genetic control of the phenomenon was studied further by using a series of recombinant inbred strains between strains STS/A and BALB/cHeA (6). Almost all the recombinant inbred strains showed intermediate values (Fig. 3). The findings proved that the response was a multiple trait and not due to one major locus in this combination of mouse strains. One CXS strain (CXS6) failed to show an IL-2 response, offering the possibility of further research into the genetic control of IL-2 response to MHV-4 infection.

IL-2 is known to be a T-cell growth factor, which is secreted from helper T cells stimulated with mitogens (5), specific antigens (3), allo and self Ia antigens (11), and phorbol ester plus calcium ionophores (19). In this study, we demonstrated that spleen cells from some MHV-infected strains of mice secreted IL-2 and IL-3 in the absence of any exogenous stimulants in vitro. For the time being, the mechanism of this phenomenon still remains obscure.

There have been some reports on the immune modification due to MHV infection. Tamura et al. (17, 18) observed an increase of brain-associated theta-positive lymphoid cells and an immunoglobulin M-immunoglobulin G switching in

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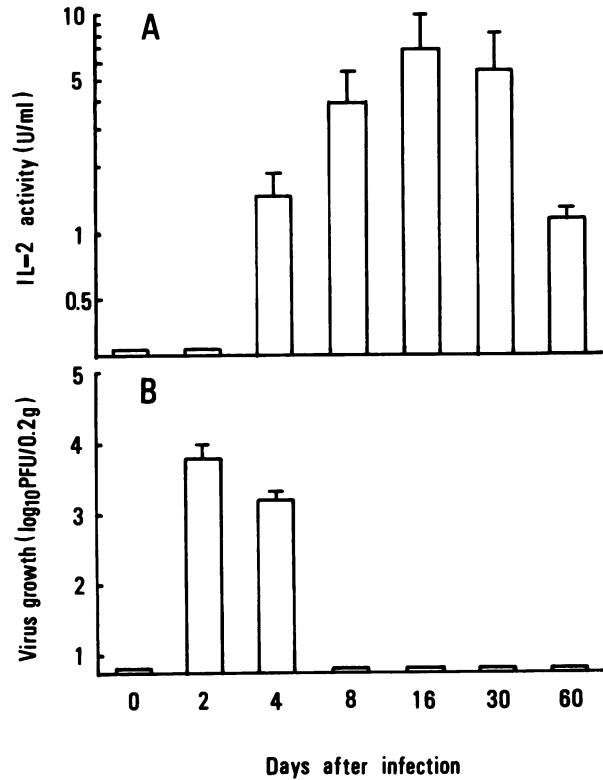
FIG. 1. IL-2 activity in the culture supernatant of spleen cells (A) and virus growth in the spleens (B) from BALB/c mice infected with MHV-4. Mean ± standard deviation from five mice.

TABLE 1. Spontaneous IL-2 production by spleen cells from MHV-infected mice of different strains

Mouse	IL-2 activity (U/ml)[a]	
	Uninfected	MHV-infected
A/J	<0.3	<0.3
BALB/cCr	<0.3	6.3 ± 0.2
BALB/cHeA	<0.3	7.9 ± 0.3
C57BL/6J	<0.3	0.8 ± 0.3
C57BL/10Sn	<0.3	1.0 ± 0.1
DBA/2Cr	<0.3	7.5 ± 0.5
STS/A	1.0 ± 0.1	40.7 ± 4.3

[a] Mean ± standard deviation from three mice.

suggested that the so-called E2 protein of MHV-4 induces Ia antigen expression, which is perhaps involved in this immune disorder as in other cases of autoimmune disease (1, 16).

MHV-4 infection in mice has been studied as a possible animal model of human demyelinating disease in the central nervous system, such as multiple sclerosis; however, the pathogenesis of demyelination after MHV-4 infection remains unclarified. After intracerebral infection with a low dose of the virus, STS/A mice showed clinical signs of generalized encephalitis and approximately one-third of them died within 2 weeks. All the surviving mice recovered asymptomatically, while half began to show severe hind leg paralysis at 1 month p.i. and died by 2 months p.i. (unpublished data). The observation was limited in this strain and was very unique in murine infection with wild-type MHV-4. Recently, Saneto et al. (13) indicated that IL-2 mediated the inhibition of oligodendrocyte progenitor cell proliferation in vitro. The findings suggest a possibility that some lymphokine(s) regulates demyelination and remyelination. Taken together, abnormal lymphokine production might be included in the pathogenesis of demyelinating disease induced by MHV-4.

the T-cell-dependent antibody response in nude mice infected with a low-virulence strain of MHV. These findings suggest that MHV infection plays a role in T-cell growth and/or differentiation, perhaps correlated with the lymphokine production observed in this study. Massa et al. (12)
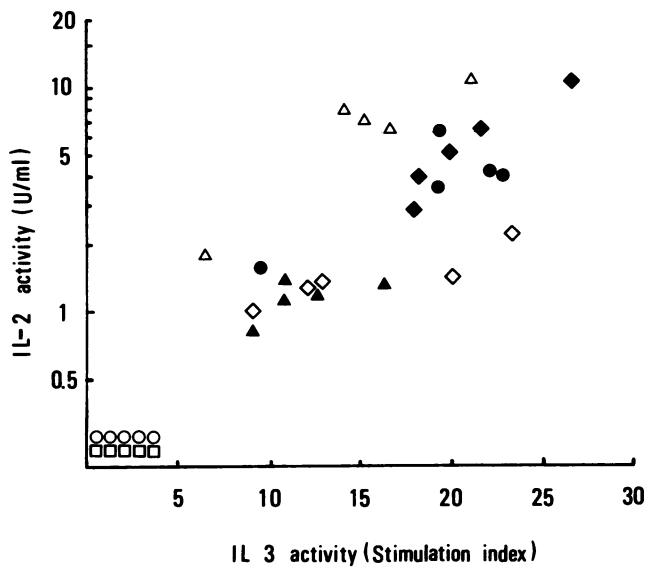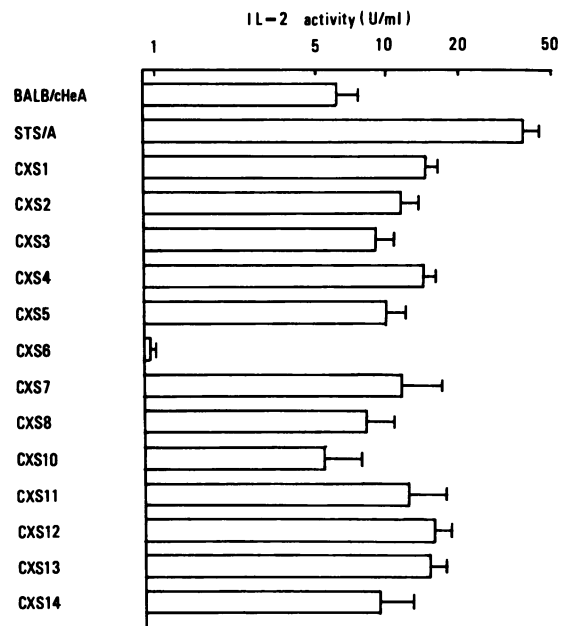

FIG. 2. IL-2 and IL-3 activities in spleen cell cultures from uninfected BALB/c mice (□) and infected mice 2 (O), 4 (◇), 8 (●), 16 (△), 30 (◆), and 60 (▲) days p.i.

FIG. 3. Spontaneous IL-2 production by spleen cells from MHV-infected recombinant inbred strains between strains STS/A and BALB/cHeA. Mean ± standard deviation from five mice.

In addition to the immune disorder observed in the subacute phase of MHV infection, severe but transient immunosuppressions were reported in the acute phase (2, 14). Since MHV is one of the most common viruses infecting laboratory mice, it is clearly important to use laboratory mice of known microbiological status for immunologic experiments.

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