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Induction of Self-Reactive T Cells after Murine Coronavirus Infection

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We studied the mechanism of in vitro spontaneous lymphokine production by spleen cells from mice injected intraperitoneally with murine coronavirus strain JHM 1 month after infection, when infectious virus had already been cleared from the spleens. Removal of either $CD4^+$ T cells or Ia^+ antigen-presenting cells (APC) from the spleen cells abrogated interleukin-2 (IL-2) production. Addition of anti-CD4 or anti-Ia^d monoclonal antibodies to the culture suppressed IL-2 production. These results suggest that the response involved typical receptor-mediated activation of T cells. Surprisingly, reciprocal mixing experiments with a coculture of T cells from infected mice and APC from either infected or naive mice resulted in the production of IL-2. The absence of viral antigens in spleen cells 1 month after infection, as indicated by their inability to induce the proliferation of T-cell clones specific for the viral antigens, suggest that the T cells from mice 1 month after infection were not responding to the viral antigens. The inoculum components other than the virus did not induce this immune response. We also found that the frequency of self-reactive but not alloreactive IL-2-producing T cells in the spleens of infected mice was 3- to 10-fold higher than that in naive mice. These findings suggest that an increased frequency of self-reactive T cells which secrete IL-2 occurs following murine coronavirus infection. This may have important implications in the development of autoimmunelike phenomena following murine coronavirus infection.

Virus infections can perturb the immune system in various ways. Immunosuppression due to infection of immunocompetent cells has been observed in a number of virus infections in humans and in animals (31, 35). Typically, AIDS in humans ensues following the loss of $CD4^+$ T cells after infection with human immunodeficiency virus (32). In addition, impaired T-cell-mediated immunity may result from virus-induced modulation of expression of major histocompatibility complex (MHC) antigens (14), since T cells recognize foreign antigens only in the context of self-MHC molecules. On the other hand, aberrant immune responses following some viral infections are suspected to trigger autoimmune diseases (37, 54). In such cases, "molecular mimicry," by which an infectious agent shares a determinant with a host protein, results in the stimulation of T cells and/or B cells against the cross-reactive host protein and causes disease. Furthermore, upregulation of MHC antigen expression induced by virus infection may contribute to aggravation of the disease (30, 48). Thus, the interaction between viruses and the host's immune system is complicated, and much remains to be elucidated.

Infection of rodents with a neurotropic murine coronavirus, strain JHM (MHV-4), has been extensively studied as a model of virus-induced diseases in the central nervous system (9, 30, 48, 52, 53). MHV-4 induces acute encephalitis and subacute paralysis in mice that survive infection. We previously reported an interesting immune response in mice following MHV-4 infection (24). Briefly, spleen cells from some strains of mice infected intraperitoneally with MHV-4 but not those from naive mice produce a significant amount of interleukin-2 (IL-2) and IL-3 in the absence of exogenous stimulants in vitro. This response is detectable for quite a long time, from 4 to 60 days after infection.

Since these lymphokines are secreted by activated $CD4^+$ T cells following stimulation with specific antigen in the presence of Ia^+ antigen-presenting cells (APC) (54), it was initially assumed that these lymphokines were secreted by virus-specific T cells activated by viral antigens. We were, however, much interested in why the response was detectable for such a long time after the clearance of virus from the spleens, since infectious virus is usually eliminated from the spleens within a week after intraperitoneal infection and viral antigens are undetectable 1 month after infection. In this study, we focused on the mechanism of the spontaneous lymphokine production 1 month after infection and found that the response involved normal T-cell receptor-mediated T-cell activation events. However, since viral antigen was not detected even by virus-specific T-cell clones, and the inoculum components other than the virus did not induce this immune response, we suggest that T cells from mice 1 month after infection may be stimulated by self-antigens. We also found that the frequency of self-reactive but not alloreactive IL-2-producing T cells in infected mice was 3- to 10-fold higher than that in naive mice. These results suggest that MHV-4 infection can induce an autoimmune T-cell response in mice.

MATERIALS AND METHODS

Mice. BALB/c mice were bred in our facilities. (BALB/c × C57BL/6)F_1 and (BALB/c × DBA/2)F_1 mice were purchased from Charles River Japan, Atsugi, Japan. All the mice were kept in semibarrier conditions and used at the age of 6 to 12 weeks. The mouse breeding colonies had been routinely checked serologically for the absence of MHV-4 (24).

Virus. MHV-4 was propagated in serum-free conditions, assayed for PFU on DBT cells as described elsewhere (24), and stored at −70°C until use. The virus used in this study

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did not have determinants characterized by monoclonal antibody (MAb) J.7.2 or J.2.2 (8) (data not shown).

Antibodies. Anti-Thy-1.2 MAb was purchased from Serotec, Bicester, U.K. Anti-Lyt-1.2 and anti-Lyt-2.2 MAbs were obtained from Cederlane, Ontario, Canada. Ascites fluids of anti-mouse MHC class I (anti-K^d and anti-D^d) MAb and anti-mouse MHC class II (anti-Ia^d) MAb were obtained from the Meiji Institute of Health, Tokyo, Japan. Another anti-class II (anti-$IA^{k,s,f}$) MAb (MAb 10-2.16) was purchased from Litton Bionetics, Kensington, Md. Rat hybridomas GK1.5 (anti-CD4) and 7D4 (anti-IL-2 receptor) were obtained from the American Type Culture Collection, Rockville, Md. Biotin-conjugated anti-Thy-1.2 and anti-Lyt-2 MAbs were purchased from Becton Dickinson, Mountain View, Calif. In some experiments, MAbs M5/114.2 (anti-$IA^{b,d,q}$,$IE^{d,k}$), RL172.4 (anti-CD4), and 31M (anti-CD8) were also used (2, 3, 44).

Assay for spontaneous IL-2 production by spleen cells from BALB/c mice after MHV-4 infection. The methods for the spleen cell assay were described previously (24). Briefly, female 6-week-old BALB/c mice were inoculated intraperitoneally with 10^4 PFU of MHV-4. One month later, spleens were removed aseptically and a single-cell suspension at a concentration of 5×10^6/ml was prepared in RPMI 1640 medium (GIBCO, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal calf serum (FCS), 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 5×10^{-5} M 2-mercaptoethanol, and antibiotics. After incubation at 37°C for 48 h under 5% CO_2 in air, the IL-2 activity of the supernatant was quantitated by use of the IL-2-dependent CTLL-2 cell line.

Separation of spleen cells. For negative selection, spleen cells were treated with various MAbs plus complement in a standard procedure (26). Spleen cells were fractionated by passage through a Sephadex G-10 column (Pharmacia, Uppsala, Sweden) or nylon-wool column (Wako, Tokyo, Japan) by the methods of Ly et al. (27) and Julius et al. (15), respectively.

In reconstruction experiments, a T-cell fraction was prepared from spleen cells by passage through a nylon-wool column. The APC fraction was obtained from spleen cells treated with a combination of anti-Thy-1.2 and anti-CD4 MAbs plus complement.

Antigen-dependent proliferation assay with MHV-4-specific T-cell clones. Three MHV-4-specific T-cell clones, 1.42, 1.12, and 2.35, were established, maintained as described previously (25), and used in the antigen-dependent proliferation assay at 7 days after the last antigen stimulation. One million spleen cells from BALB/c mice 1 month after MHV-4 infection and from naive mice were gamma-irradiated and added to each well containing 2×10^4 T-cell clones in 0.2 ml of RPMI 1640 medium supplemented with 10% FCS, 25 mM HEPES, 5×10^{-5} M 2-mercaptoethanol, and antibiotics. For the positive control, these spleen cells were infected with MHV-4 in vitro and then cultured with the T-cell clones. Cells were pulse-labeled at 40 h with 1 μCi of [^{3}H]thymidine and then incubated for additional 8 h.

Flow cytometric analysis. Cell surface staining was carried out and analyzed on a flow cytometer as described previously (25).

Limiting-dilution assay for IL-2-producing cells. Frequencies of IL-2-producing T cells were estimated by limiting-dilution analysis (26, 34) with a minor modification. Each well of V-bottomed 96-well plates contained 10^4 T-cell-depleted resident peritoneal cells and various numbers of T cells in 0.2 ml of RPMI 1640 medium supplemented with 10% FCS, 25 mM HEPES, 5×10^{-5} M 2-mercaptoethanol, and antibiotics. After a 5-day incubation at 37°C, 100 μl of the supernatants was removed from each well and transferred into a well containing 4,000 CTLL-2 cells in a flat-bottomed 96-well plate. After 20 h of incubation, 0.5 μCi of [^{3}H]thymidine was added to each well and incubated for a further 4 h. A typical titration included 24 replicate wells at each of four or five responder cell concentrations, together with 24 wells which contained resident peritoneal cells only, to provide a measure of background IL-2 production. Every assay included a reference IL-2 standard to get a standard curve. Wells were scored positive for IL-2 production if the number of cpm in the IL-2 assay was greater than 3 standard deviations (SDs) above the mean cpm of the background. Data were analyzed by the reiterative maximum-likelihood methods of Porter and Berry (41). Calculations were performed with an NEC PC-9801 computer with a program written in BASIC.

TABLE 1. Characterization of cells involved in spontaneous IL-2 production by spleen cells from BALB/c mice 1 month after MHV-4 infection[a]

Expt no.	Treatment	Cells negatively selected	IL-2 activity (U/ml)
1	None		6.0
	C		3.4
	Anti-Thy-1.2 + C	T cells	<0.3
	Anti-Lyt-1.2 + C	$CD5^+$ T cells	<0.3
	Anti-Lyt-2.2 + C	$CD8^+$ T cells	5.0
	Passage through nylon-wool column	Macrophages and B cells	<0.3
2	None		5.2
	C		4.3
	Anti-CD4 + C	$CD4^+$ T cells	<0.3
	Anti-Ia + C	Ia^+ cells	<0.3
	Passage through G-10 column	Macrophages	1.6
	Passage through nylon-wool column	Macrophages and B cells	<0.3

[a] Spleen cells from BALB/c mice 1 month after MHV-4 infection were treated with MAb plus complement (C) or fractionated on a nylon-wool column and G-10 column. Viable cells at a concentration of 5×10^6/ml were cultured, and IL-2 activity in the supernatant was measured as described in Materials and Methods.

RESULTS

Characterization of cells involved in spontaneous IL-2 production 1 month after infection. Spleens were removed from BALB/c mice 1 month after intraperitoneal infection with MHV-4. To characterize the cell types involved, the spleen cells were treated with various MAbs plus complement or fractionated on a nylon wool column or Sephadex G-10 column to deplete specific subsets of cells before culture. Table 1 shows that the depletion of either $Thy\text{-}1^+$, $Lyt\text{-}1^+$, $CD4^+$, or Ia^+ cells or removal of nylon wool-adherent cells completely abrogated lymphokine production. Passage through the G-10 column partially decreased the response. Depletion of $CD8^+$ cells did not influence the level of IL-2 production. These results suggest that the response involves at least two cell types, $CD4^+$ T cells and Ia^+ APC, and possibly macrophages and B cells, as is the case with any T-cell activation event (54).

Effect of various MAbs on spontaneous lymphokine production by spleen cells from BALB/c mice 1 month after MHV-4

TABLE 2. Effect of MAbs on spontaneous IL-2 production by spleen cells from BALB/c mice 1 month after MHV-4 infection

MAb[a]	IL-2 activity (U/ml)	% Inhibition vs control[b]
None (control)	12.3	0
Anti-Ia^d	<0.3	>97
Anti-K^d	22.8	0
Anti-D^d	10.0	19
Anti-IA^k (10-2.16)	12.3	0
Anti-CD4 (GK1.5)	<0.3	>97
Anti-CD4 (RL172.4)	0.7	94
Anti-CD8 (31M)	18.0	0

[a] Ascites and culture supernatants of MAbs were used at a concentration of 1% and 6%, respectively.
[b] Percent inhibition was calculated by comparison with the IL-2 activity of the supernatant without antibody.

infection. To examine the interaction between T cells and APC during in vitro culture, various MAbs were added to the spleen cell suspension before culture. Table 2 shows that the anti-Ia^d but not the anti-IA^k MAb effectively inhibited IL-2 production. In addition, both anti-CD4 MAbs (GK1.5 and RL172.4) but not the anti-CD8 (31M) MAb completely suppressed spontaneous IL-2 production by spleen cells from MHV-4-infected mice. These results suggest that IL-2 secretion 1 month after infection is a consequence of T-cell activation events involving accessory CD4 and Ia glycoproteins.

Coculture of T cells with APC from naive and infected BALB/c mice. To confirm the hypothesis described above, we carried out reciprocal mixing experiments with nylon wool-purified T cells and APC from infected or naive BALB/c mice. As expected, nylon wool-purified T cells from MHV-4-infected mice produced IL-2 after coculture with APC from MHV-4-infected mice (Table 3). Surprisingly, IL-2 production was also observed with APC from naive mice. No IL-2 was detected in the culture supernatant of T cells in the absence of APC. Furthermore, APC from both MHV-4-infected and naive mice also failed to stimulate T cells from naive mice. These findings fit the idea that lymphokine production is a consequence of T-cell activation in the presence of APC. The data also suggest that lymphokine production in the apparent absence of exogenous antigen is due to abnormal activation of T cells from infected mice, since APC from both infected and naive mice were stimulatory.

TABLE 3. Reconstruction of T cells and APC from BALB/c mice 1 month after MHV-4 infection

Source of cells[a]		IL-2 activity[b] (U/ml)
T cells	APC	
MHV-4	MHV-4	3.2
	Naive	4.4
	None	<0.3
Naive	MHV-4	<0.3
	Naive	<0.3
	None	<0.3
None	MHV-4	<0.3
	Naive	<0.3

[a] T cells were purified from spleens of MHV-4-infected or naive BALB/c mice by passage through a nylon-wool column twice. APC from MHV-4-infected or naive mice were obtained by treating spleen cells with a combination of anti-Thy-1.2 and anti-CD4 MAbs plus complement.
[b] Equal volumes of T-cell suspensions and APC suspensions at a concentration of 5×10^6/ml were mixed and cultured for 48 h. IL-2 activity in the supernatant was measured as described in Materials and Methods. Similar results were obtained in three separate experiments.

TABLE 4. MHV-4-specific T-cell clones do not proliferate in response to spleen cells from BALB/c mice 1 month after MHV-4 infection[a]

T-cell clone	Source of stimulator cells	Infection in vitro	Mean [^{3}H]thymidine uptake[b] (cpm) ± SD
1.42	Naive	−	4,995 ± 1,397
		+	41,352 ± 5,189
	MHV-4	−	6,428 ± 2,765
		+	45,632 ± 2,030
	None		540 ± 93
1.12	Naive	−	397 ± 80
		+	135,766 ± 5,478
	MHV-4	−	2,084 ± 1,475
		+	131,877 ± 5,368
	None		3,354 ± 2,481
2.35	Naive	−	3,406 ± 1,713
		+	93,889 ± 3,219
	MHV-4	−	1,412 ± 530
		+	99,354 ± 2,289
	None		893 ± 514
None	Naive	−	1,183 ± 562
		+	828 ± 541
	MHV-4	−	958 ± 1,003
		+	694 ± 320

[a] 2×10^4 T-cell clones were incubated with 10^6 gamma-irradiated spleen cells from MHV-4-infected or naive BALB/c mice, with or without infection in vitro.
[b] Results are presented as the mean ± SD of a triplicate assay. Similar results were obtained in another experiment.

Specificity of spontaneous lymphokine production 1 month after infection. We used MHV-4-specific CD4$^+$ T-cell clones to assay for the presence of MHV-4 antigens. These T-cell clones secrete IL-2 upon stimulation with MHV-4 antigen and the appropriate APC. Since viral antigens in the spleens 1 month after intraperitoneal infection are undetectable by immunofluorescence or immunoperoxidase staining, we used the T-cell clones to test for the presence of MHV-4 antigens in the spleens from BALB/c mice 1 month after MHV-4 infection by determining whether these cells could induce the proliferation of MHV-4-specific T-cell clones. None of three MHV-4-specific T-cell clones responded to spleen cells from infected BALB/c mice in the absence of exogenous MHV-4 (Table 4). These data strongly suggest that MHV-4 antigens, as with infectious virus, are not detectable in the spleens of mice 1 month after infection. This suggests that IL-2 secretion by T cells 1 month after infection is unlikely to be induced by MHV-4 antigens.

Two experiments were designed to confirm the absence of all viral antigens from spleen cells 1 month after infection. Spleen cells from infected mice 1 month after infection and from naive mice were cultured in the presence or absence of UV-treated MHV-4, and the IL-2 activity of the supernatants was measured (Table 5). The addition of inactivated MHV-4 did not induce IL-2 secretion by naive spleen cells, suggesting that MHV-4 proteins do not have mitogenic activity on murine T cells. The same treatment, however, slightly enhanced IL-2 production by spleen cells from infected mice. The increase in IL-2 production may be

TABLE 5. Effect of UV-treated MHV-4 on spontaneous IL-2 production by spleen cells from mice 1 month after infection[a]

Source of spleen cells	UV-treated MHV-4 (μg/ml)	IL-2 activity (U/ml)
MHV-4	100	10.5
	50	9.8
	10	9.7
	0	6.1
Naive	100	<0.3
	50	<0.3
	10	<0.3
	0	<0.3

[a] Spleen cells from either BALB/c mice 1 month after MHV-4 infection or naive mice were cultured in the presence of UV-treated MHV-4. The uninfectivity of UV-treated MHV-4 was confirmed on DBT cells.

interpreted as a result of IL-2 secretion by MHV-4-reactive T cells which were generated in the spleens of mice 1 month after infection and is consistent with the above hypothesis, that spontaneous IL-2 secretion by T cells 1 month after infection is MHV-4 antigen independent.

We examined whether addition of antiviral antibodies to the spleen cell culture inhibited spontaneous IL-2 production. Although anti-MHV-4 mouse serum decreased IL-2 production by spleen cells from mice 1 month after infection, the same level of decrease was also observed with normal mouse serum (Fig. 1). These data indicate that antiviral antibodies do not inhibit IL-2 production even though mouse serum itself tends to suppress the proliferation of CTLL-2 cells.

One might argue that this immune response is specific for serum or some component of the inoculum. Although we do not use any serum in the propagation of virus, some experiments were carried out to rule out this possibility. First, spleen cells from mice injected with culture supernatant from uninfected DBT cells did not produce IL-2 in vitro (<0.3 U of IL-2 per ml), whereas those from mice injected with an equal volume of MHV-4-containing inoculum did (6.3 ± 0.3 U/ml [mean ± SD, n = 5]). These results suggest

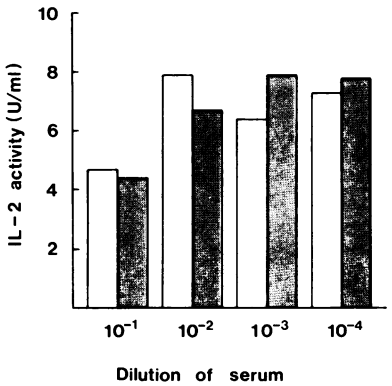

FIG. 1. Effect of antiviral antibody on spontaneous IL-2 production by spleen cells from mice 1 month after MHV-4 infection. The spleen cells were cultured in the presence of hyperimmune mouse serum (open columns) and normal mouse serum (shaded columns), and the IL-2 activity in the supernatants was measured. Virus-neutralizing titers of hyperimmune and normal mouse sera were 1:27,000 and >1:10, respectively. The IL-2 activity of the supernatant without mouse serum was 6.8 U/ml in this experiment. Similar results were obtained in another experiment.

TABLE 6. Surface profile of spleen cells from MHV-4-infected and naive BALB/c mice[a]

Mice	Mean % of cells positive ± SD[b]			
	Thy-1.2	CD4	CD8	IL-2 receptor
MHV-4 infected	42 ± 5	23 ± 6	18 ± 1	<2
Naive	46 ± 6	24 ± 5	19 ± 2	<2

[a] Spleen cells from BALB/c mice 1 month after MHV-4 infection and those from naive mice were analyzed by flow cytometry.
[b] Mean ± SD for five mice.

that this immune response is clearly associated with the virus infection. Since FCS has been suspected to be a source of antigens which stimulate "autoreactive" T cells (42), we used 1% fresh mouse serum instead of 10% FCS for the in vitro spleen cell culture and detected some but less IL-2 activity (6.9 versus 1.7 U/ml for 10% FCS and 1% normal mouse serum, respectively). Therefore, this immune response seems to be specific for some kind of self-antigens.

Attempts to characterize the aberrancy of T cells from MHV-4-infected mice. The expression of some cell surface markers (Thy-1.2, CD4, CD8, and IL-2 receptor) on spleen cells from MHV-4-infected mice was examined and compared with that of naive mice, because the drop in the $CD4^+/CD8^+$ cell ratio might be due to an increase in $CD4^+$ helper T cells and/or a decrease in $CD8^+$ suppressor T cells. However, no significant difference was observed in the expression of the markers tested (Table 6).

We compared precursor frequency of IL-2-producing T cells in splenic T cells from mice 1 month after MHV-4 infection and from naive mice after stimulation with self-APC by limiting-dilution analysis (Fig. 2). The precursor frequency of self-reactive T cells in naive mice was estimated at approximately 1 in 42,000. In contrast, that in MHV-4-infected mice was shown to be approximately 1 in 4,000 (Table 7, experiment 1). The precursor frequency of alloreactive and Mls-1^a-reactive T cells in these mice was also estimated by stimulating T cells with APC from (BALB/c × C57BL/6)F_1 and (BALB/c × DBA/2)F_1 mice, respectively. No differences in the frequencies of alloreactive and Mls-1^a-reactive IL-2-producing T cells were ob-

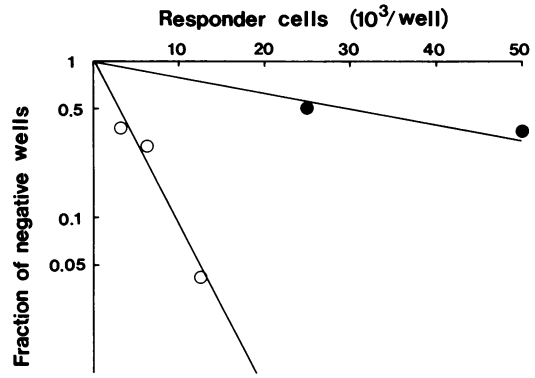

FIG. 2. Limiting-dilution analysis of IL-2-producing cells in splenic T cells from BALB/c mice 1 month after MHV-4 infection (○) and from naive mice (●) after stimulation with self-APC. Mean cpm ± SD for 24 wells containing self-APC only was 328.8 ± 110.3. Wells were scored positive if the number of cpm was greater than 660 cpm in this experiment.

TABLE 7. Limiting-dilution analysis of self-reactive IL-2-producing T cells in spleens from MHV-4-infected and naive BALB/c mice

Expt no.	Source of T cells[a]	Source of stimulator cells[b]	Major stimulatory antigen	1/f (95% C.L.)[c]	P
1	MHV-4	BALB/c	Self-antigens	4,055 (3,027–5,495)	0.9
	Naive	BALB/c	Self-antigens	42,459 (28,576–63,076)	0.5
2	MHV-4	BALB/c	Self-antigens	4,125 (2,845–5,973)	0.3
	Naive	BALB/c	Self-antigens	10,297 (7,273–14,577)	0.9
3	MHV-4	BALB/c	Self-antigens	3,067 (2,057–4,572)	0.2
	Naive	BALB/c	Self-antigens	11,899 (8,464–16,727)	0.3
	MHV-4	(BALB/c × C57BL/6)F_1	H-2^b	667 (499–892)	0.8
	Naive	(BALB/c × C57BL/6)F_1	H-2^b	657 (491–878)	0.5
	MHV-4	(BALB/c × DBA/2)F_1	Mls-1^a	184 (138–246)	0.2
	Naive	(BALB/c × DBA/2)F_1	Mls-1^a	213 (158–288)	0.9

[a] T cells were purified from spleen cells of MHV-4-infected or naive BALB/c mice by passage through a nylon-wool column.
[b] 10^4 T-cell-depleted peritoneal cells from naive mice were used as stimulator cells.
[c] 1/f, Reciprocal frequency; 95% C.L., 95% confidence limits.

served in MHV-4-infected and naive mice (experiment 3). These results clearly indicate that only the frequency of self-reactive T cells is increased by MHV-4 infection.

DISCUSSION

We studied the immunological mechanism of spontaneous in vitro lymphokine production by spleen cells from mice 1 month after intraperitoneal MHV-4 infection, when infectious virus has already been cleared from the spleens. Generally, a subset of $CD4^+$ T cells (Th1) secrete IL-2 after recognition of antigen by the T-cell receptor, in the context of the self-Ia (MHC class II) molecule on the APC. Signal transduction by receptor-ligand binding usually involves an accessory CD4 molecule (54). In this study, we have shown that the response involved $CD4^+$ T cells and Ia^+ APC and was blocked by anti-CD4 as well as anti-Ia MAbs. These results suggest that this immune response involves normal T-cell receptor-mediated activation events. However, reciprocal mixing experiments indicated that T cells from infected mice secreted IL-2 after stimulation not only with APC from infected mice but also with APC from naive mice. Moreover, IL-2 secretion occurred in the absence of viral antigens, as evidenced by the inability of spleen cells from infected mice to induce the proliferation of MHV-4-specific T-cell clones. These findings suggest that T cells from mice 1 month after infection are not activated by viral antigens.

The characteristics observed in spontaneous IL-2 production following MHV-4 infection bear a striking resemblance to those of self-reactive T cells. Although reports on the establishment of self-reactive T cell hybridomas and clones have been accumulating (6, 42, 43), whether they are truly self-reactive or merely responding to components of the culture medium is a matter of controversy. In particular, FCS, which is advantageous to cell culture, has been suspected to function as an antigen for some "self-reactive" T-cell hybridomas and clones (42), because they became established after long-term culture in FCS-containing medium. Unlike these cell lines, the IL-2-secreting T cells in this phenomenon were not exposed to FCS before the onset of IL-2 production. Furthermore, it is unlikely that inoculum components except for virus trigger spontaneous IL-2 production, because spontaneous IL-2 production was not observed in spleen cells from mice injected with uninfected DBT cell culture supernatant. Truly self-reactive T cells which were perhaps induced by virus infection are considered to be responsible for the spontaneous lymphokine production 1 month after MHV-4 infection, when virus has been eliminated from the spleens.

Self-reactive T cells are thought to be physiologically deleted or to be normally inactivated or suppressed (54, 56). Indeed, Kappler et al. indicated that almost all the T cells bearing $V_\beta 17a$, which are reactive for an unknown B-cell-specific product, and the IE molecule are eliminated from the T-cell repertoire during T-cell maturation in the thymus (16). The same phenomenon has been also discovered in T cells specific for Mls-1 antigen (17, 28). On the other hand, autoimmune T cells specific for myelin basic protein, for example, can be readily isolated from immunized mice, suggesting that some self-reactive T cells are inactivated or suppressed rather than deleted (54, 55). At present, the nature of the self-antigen(s) responsible for stimulating the self-reactive T cells induced after MHV-4 infection remains undefined.

IL-2 production by spleen cells from MHV-4-infected mice without exogenous stimulants was observed from 4 to 60 days after infection (24). Although we suggest that the spontaneous IL-2 production 1 month after MHV-4 infection is an autoimmune response, this might not be the case at early times of infection. Because viral antigens abound in the spleens in the acute phase, virus-specific Th1 cells could secrete IL-2 in response to the viral antigens. This mode of lymphokine production may be generally observed in various infections. Indeed, a recent study indicates the activation of IL-2 transcription in T cells during acute lymphocytic choriomeningitis virus infection (18).

Next, we attempted to identify abnormalities in T cells from mice 1 month after infection. No significant changes in expression of T-cell surface markers were observed. However, the frequency of self-reactive but not alloreactive IL-2-producing T cells in infected mice was 3- to 10-fold higher than that in naive mice. Although the T-cell receptor usage of the virus-induced self-reactive T cells is interesting, it might be difficult to analyze this with a bulk spleen population because of the low frequency of responding cells. Such a study will need some amplification, for example, establishment of self-reactive T-cell clones from spleens of infected mice.

Several hypotheses may explain this autoimmune T-cell response. First, molecular mimicry may give rise to this phenomenon. If this is the case, MHV-4 infection may activate some T cells which are reactive for both viral and

self-antigens. For example, a recent study indicates molecular mimicry between the spike protein of MHV-4 and the Fc gamma receptor (38). In addition, we found that one BALB/c-derived, MHV-4-specific T-cell clone designated P9A was also stimulated by naive DBA/2 but not BALB/c spleen cells (25), indicating that this T-cell clone has dual specificity for Ia^d plus MHV-4 and for Ia^d plus $Mls\text{-}1^a$ or a minor histocompatibility antigen which is expressed in DBA/2 but not in BALB/c cells. This finding also suggests that some host-derived proteins can mimic the viral proteins. Second, MHV-4 may function as a vector expressing cellular sequences. Since coronaviruses have been found to undergo RNA recombination at a very high frequency (19, 29), it is likely that MHV-4 may incorporate cellular RNA during replication and induce an autoimmune response by expressing the host-derived peptide. In fact, some RNA viruses have been found to have an insertion of host-derived RNA (20, 36). Third, the breakdown of the suppressor circuit may increase the activity of potentially self-reactive T cells. However, we have no evidence to support this hypothesis. Fourth, the increase in self-reactive T cells after MHV-4 infection can break down self-tolerance.

The most interesting speculation for this hypothesis is that MHV-4 infects thymic epithelial cells, suppresses Ia antigen expression on the cells, and leads to incomplete deletion of T cells expressing T-cell receptor reactive to self-antigens, as reported after repeated cyclosporine treatment (9, 13). In fact, Knobler et al. observed virus particles in thymic epithelial cells after MHV-4 infection (21). It is also well known that MHV infection shuts off cellular protein synthesis (12, 46). In addition, MHV can affect bone marrow cells (23, 42). Therefore, it is possible that MHV-4 infection creates a situation similar to that observed after repeated cyclosporine treatment following syngeneic bone marrow cell transfer, which results in reduction of Ia antigen expression on thymic epithelial cells (4). This results in incomplete deletion of T cells bearing T-cell receptor reactive to self-antigen (9, 13) and the induction of T-cell-dependent, organ-specific autoimmune diseases (10, 11, 47). Finally, it has been reported that MHV infection induces T-cell growth and/or differentiation in athymic nude mice (33, 45, 50, 51). Although the mechanism of this phenomenon is unknown, such a population of T cells might contain self-reactive T cells because the T cells have not been "educated" by the thymus. Therefore, MHV-promoted T-cell induction might explain the induction of self-reactive T cells following MHV-4 infection if this phenomenon occurred in euthymic mice.

Although the relevance of autoimmunity to viral pathogenesis is most interesting, such an issue remains poorly understood. It is suggested that self-reactive T cells regulate immune responses by secreting a variety of lymphokines and may therefore play a role in the development of some autoimmune diseases (56). Watanabe et al. first reported that MHV-4 infection can induce an autoimmune T-cell response (52). They showed that lymphocytes from MHV-4-infected Lewis rats are responsive to myelin basic protein and that stimulated lymphocytes can transfer a mild clinical disease to recipients. However, it is presently unclear what role self-reactive T cells play in MHV-4-infected mice. Central nervous system disease is rarely seen in mice following intraperitoneal MHV-4 infection, even though a self-reactive T-cell response is induced. However, while no clinical disease was observed in BALB/c mice, STS/A mice, which produced higher levels of IL-2, showed evidence of skin lesions. There have been several reports of MHV infections increasing the resistance of mice to other unrelated pathogens or malignancies (1, 5, 7, 22). The self-reactive T cells produced following MHV infection might contribute to augmented protection in a nonspecific fashion.

Since virus-induced autoimmunity may involve varied and complex molecular interactions between virus and host, as hypothesized above, many attempts should be made to study it. We believe that autoimmune T-cell responses following MHV-4 infection provide a unique opportunity to elucidate the phenomenon.

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