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Virus and Autoimmunity: Induction of Autoimmune Disease in Mice by Mouse T Lymphotropic Virus (MTLV) Destroying CD4^{+} T Cells[1]

Stephen S. Morse,* Noriko Sakaguchi,[†] and Shimon Sakaguchi[2†]

Neonatal infection of the mouse T lymphotropic virus (MTLV), a member of herpes viridae, causes various organ-specific auto-immune diseases, such as autoimmune gastritis, in selected strains of normal mice. The infection selectively depletes CD4^{+} T cells in the thymus and periphery for 2–3 wk from 1 wk after infection. Thymectomy 3 wk after neonatal MTLV infection enhances the autoimmune responses and produces autoimmune diseases at higher incidences and in a wider spectrum of organs than MTLV infection alone. On the other hand, inoculation of peripheral CD4^{+} cells from syngeneic noninfected adult mice prevents the autoimmune development. These autoimmune diseases can be adoptively transferred to syngeneic athymic nude mice by CD4^{+} T cells. The virus is not detected by bioassay in the organs/tissues damaged by the autoimmune responses. Furthermore, similar autoimmune diseases can be induced in normal mice by manipulating the neonatal thymus/T cells (e.g., by neonatal thymectomy) without virus infection. These results taken together indicate that neonatal MTLV infection elicits autoimmune disease by primarily affecting thymocytes/T cells, not self Ags. It may provoke or enhance thymic production of CD4^{+} pathogenic self-reactive T cells by altering the thymic clonal deletion mechanism, or reduce the production of CD4^{+} regulatory T cells controlling self-reactive T cells, or both. The possibility is discussed that other T cell-tropic viruses may cause autoimmunity in humans and animals by affecting the T cell immune system, not the self Ags to be targeted by the autoimmunity. *The Journal of Immunology,* 1999, 162: 5309–5316.

T he virus is the most plausible etiologic agent of autoimmune disease, but which virus or how it causes autoimmune disease is largely obscure at present (reviewed in Refs. 1–3). Viruses may infect a particular organ/tissue, and elicit autoimmune responses to it through bystander activation of self-reactive T cells or by causing a leakage of sequestered self Ags, modifying the antigenicity of self molecules, or triggering their presentation to potentially self-reactive T cells through aberrant expression of MHC molecules (4–9). They may also antigenically mimic self molecules (10, 11). Although extensive attempts have been made to prove these mechanisms, evidence is still tenuous and indirect. As an alternative mechanism of virus-induced autoimmune disease, viruses may infect cells composing the immune system, thereby altering central or peripheral control on self-reactive lymphocytes (12–16). In this study, we show that a T cell-tropic virus indeed causes autoimmune disease in normal mice by infecting thymocytes/T cells, apparently not the organs/tissues to be targeted in the autoimmune disease. The autoimmune diseases thus induced are immunopathologically similar to the counterparts in humans.

T cells are key mediators of many organ-specific autoimmune diseases, such as autoimmune thyroiditis, gastritis with pernicious anemia, and insulitis in insulin-dependent diabetes mellitus (IDDM),[3] in humans and animals (1). T cells may also play key roles in systemic autoimmune diseases, such as systemic lupus erythematosus, by polyclonally activating B cells (1). To maintain immunologic self tolerance, these pathogenic self-reactive T cells must be eliminated in the thymus or, when produced and released by the thymus, their expansion/activation must be controlled in the periphery. Autoimmune disease may develop when exogenous insults, such as virus infection, affect the thymus and elicit or enhance the production of pathogenic self-reactive T cells, or prepare immunologic conditions favorable to their peripheral activation and expansion, or both (17, 18). This study shows that neonatal infection of the mouse T lymphotropic virus (MTLV) (also called thymic necrosis virus or murid herpesvirus 3) (19–22), which destroys CD4^{+} T cells in the thymus and periphery for a limited period, indeed causes autoimmune disease in selected strains of normal mice. The autoimmune development can be prevented by inoculating CD4^{+} T cells from normal syngeneic mice. Furthermore, similar autoimmune disease can be produced by directly manipulating the neonatal thymus/T cells without virus infection. MTLV thus appears to affect primarily the thymic or peripheral control of self-reactive T cells, not the organs/tissues to be targeted by the autoimmune response, thereby leading to activation and expansion of self-reactive T cells. This MTLV-induced autoimmune disease may have a common pathogenetic basis with the autoimmunities caused by other CD4 T cell-tropic viruses, including HIV (23, 24).

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[3] Abbreviations used in this paper: IDDM, insulin-dependent diabetes mellitus; HE, hematoxylin and eosin; MTLV, mouse T lymphotropic virus; Tx, thymectomy.

Materials and Methods

Mice

BALB/c mice and BALB/c *nu/nu* mice were purchased from Life Science Associates (St. Petersburg, FL), or Japan SLC (Shizuoka, Japan). To obtain newborn mice, BALB/c mice were mated in our animal facility.

Mouse T lymphotropic virus

Thymus homogenates containing MTLV were prepared as previously described (22). Virus titer was generally 3.5–4.2 $\log_{10}$ ID_{50}/ml. Fifty microliters (equivalent to 50 ID_{50}) of virus preparation were i.p. inoculated with 30-gauge needle into newborn mice within 24 h after birth (day 0). Bioassay and titration of the virus were previously described (22). Briefly, 50 μl homogenates of various tissues were i.p. inoculated into litters of newborn BALB/c mice; the thymuses of the pups were examined 10 days later. The thymuses become small and opaque in infected sucklings (21, 22).

Thymectomy (Tx)

Three-week-old mice were anesthesized by i.p. injection of pentobarbital (Abbott Laboratories, North Chicago, IL), and the thymus was removed en bloc under a dissecting microscope with forceps. As sham-Tx, the sternum was cut without removal of the thymus. The wound was sutured and the mice were kept at 30°C overnight and then returned to their mother. Neonatal thymectomy was performed as previously described (25).

Preparation of T cell subpopulations

Lymphocyte suspensions (5×10^7) prepared from spleens and lymph nodes (inguinal, axillary, brachial, and mesenteric lymph nodes) were incubated in 12×75-mm glass tubes (Corning, Corning, NY) with 100 μl of 1/10 diluted ascites of anti-L3T4 (CD4) (GK1.5, rat IgG2b) (26), anti-Lyt-2.2 (CD8) (mouse IgG2a) (27), or anti-CD25 (rat IgM) (28) for 45 min on ice, washed once with HBSS (Life Technologies, Grand Island, NY), incubated with 1 ml of nontoxic rabbit serum (Life Technologies) 1/5 diluted with Medium 199 (Life Technologies) for 30 min in a 37°C water bath with occasional vigorous shakings, added with 100 μg of DNase I (Sigma, St. Louis, MO) for the last 5 min of the incubation, and washed with HBSS, as previously described (25). To remove B cells as well as $CD4^+$ or $CD8^+$ cells completely after anti-CD4 or anti-CD8 plus C treatment, respectively, the treated cells were incubated with the J11D rat mAb (29) as culture supernatant for 30 min, washed, and incubated for 1 h at 4°C on plastic dishes precoated overnight with affinity-purified goat anti-rat IgG (Cappel-Organon Teknika, West Chester, PA), and nonadherent cells were collected (25). More than 95% of cells were positive for anti-CD4 or anti-CD8 staining after anti-Lyt-2.2 + C or anti-L3T4 + C treatment and subsequent J11D panning, respectively.

Flow-cytometric analysis

A total of 1×10^6 cells was stained with FITC anti-CD4 and PE anti-CD8, purchased from PharMingen (San Diego, CA), then analyzed by a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). To analyze the composition of T cells expressing TCRs with particular Vβ domains, cells were first incubated with anti-Vβ-6 (RR4-7) (30), Vβ-8.1, 8.2 (KJ16) (31), or Vβ-11 (RR3-15) (32) Abs (also purchased from PharMingen), washed, incubated with FITC-labeled F(ab′)$_2$ mouse anti-rat IgG (Jackson ImmunoResearch, West Grove, PA), washed, blocked with normal rat serum, and then incubated with PE anti-CD4 (PharMingen), as previously described (33).

Detection of serum autoantibodies

The ELISA (using alkaline phosphatase-conjugated secondary Ab and *p*-nitrophenyl disodium hexahydrate as the substrate) for detecting autoantibodies specific for the gastric parietal cell Ags or mouse thyroglobulins was previously described (34).

Histology and criteria for grading of autoimmune disease

Tissues and organs (thyroid, lung, pancreas, stomach, adrenal gland, kidney, ovaries, or testes) were fixed in 10% Formalin and processed for hematoxylin and eosin (HE) staining. Gastritis was graded 0 to 2+ depending on macroscopic and histologic severity: 0 = the gastric mucosa was histologically intact; 1+ = gastritis with histologically evident destruction of parietal cells and cellular infiltration of the gastric mucosa; 2+ = severe destruction of the gastric mucosa accompanying the formation of giant rugae due to compensatory hyperplasia of mucous secreting cells (see Ref. 18 for the giant rugae) (25). In immunohistochemistry, paraffin sections of gastric mucosa were stained with mAb specific for the
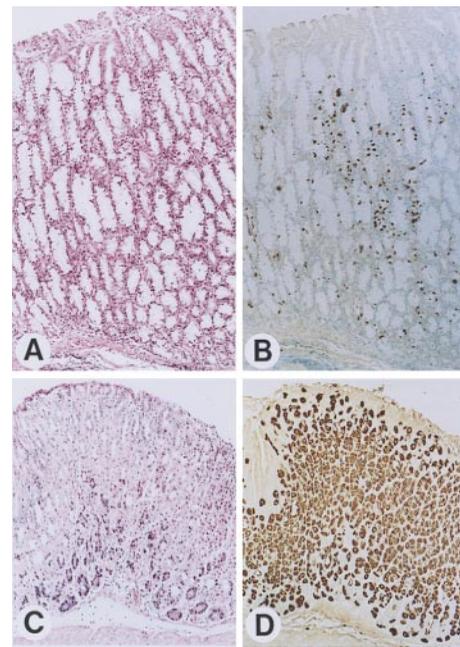

FIGURE 1. *A*, Histology of autoimmune gastritis in a neonatally MTLV-infected BALB/c mouse (HE staining, ×25). *B*, Loss and destruction of parietal cells in gastritis. Another section of the same gastric mucosa as shown in *A* was stained with monoclonal anti-parietal cell autoantibody by immunoperoxidase technique (×25). *C*, Normal gastric mucosa of a mock-infected BALB/c mouse (HE staining, ×25). *D*, Normal gastric mucosa, same as shown in *C*, with intact parietal cells demonstrated by immunoperoxidase staining with anti-parietal cell mAb (×25). Note thick gastric mucosa in *A* and *B*, compared with *C* and *D*, due to compensatory hyperplasia of mucous cells after destruction of parietal cells and chief cells in autoimmune gastritis.

gastric parietal cells, and horseradish peroxidase-labeled goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL) as the secondary reagent (33). The 4E2 hybridoma-secreting mAb (mouse IgG2a) specific for the mouse gastric parietal cells was prepared by fusing myeloma cells with B cells from a mouse bearing autoimmune gastritis and a high titer of anti-parietal cell autoantibody after neonatal cyclosporin A treatment (34).

Results

Development of autoimmune disease in selected strains of normal mice after neonatal MTLV infection

Inoculation of MTLV on day 0 or 1 produced histologically evident gastritis in 3 mo in 30–40% of BALB/c (H-2^d) and A (H-2^a), but not in C57BL/6 (H-2^b), C3H (H-2^k), or DBA/2 (H-2^d) mice (Figs. 1 and 2). In the gastritis-bearing mice, inflammatory cells, mainly mononuclear cells, infiltrated the gastric mucosa and specifically destroyed the parietal cells and chief cells, as shown by histology (Fig. 1, *A* and *C*) and immunohistochemical staining of the affected or normal gastric mucosa with a mAb specific for the gastric parietal cells (Fig. 1, *B* and *D*). Significant titers of anti-parietal cell autoantibodies were detected by ELISA in 60–80% of BALB/c, A, or C3H mice, but in few DBA/2 or C57BL/6 mice (Fig. 2). Mice with histologically evident gastritis generally showed high titers of anti-parietal cell autoantibodies. Some (approximately 10%) of the MTLV-infected A/J mice developed histologically evident oophoritis (17, 18). Autoantibodies specific for the thyroglobulin were detected by ELISA in 10–20% of MTLV-infected BALB/c or A/J mice, but anti-DNA autoantibodies were not. In contrast to MTLV infection on day 0 or 1, infection on day 7 after birth or later, or two inoculations in adults (data not shown)

FIGURE 2. Histologic severity of gastritis and titers of anti-parietal cell autoantibody in various strains of mice neonatally infected with MTLV. MTLV was inoculated on day 0 after birth, and mice were histologically and serologically examined 3 mo later. ○, Intact gastric mucosa; ◍, grade 1 gastritis; ●, grade 2 gastritis (see *Materials and Methods* for histologic grading of gastritis).
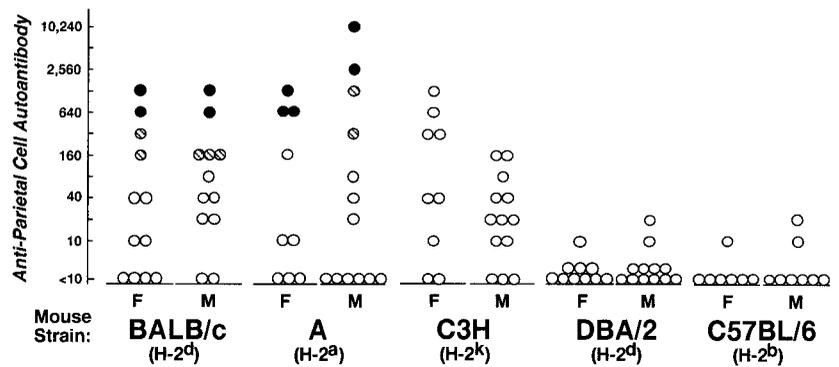
was ineffective in eliciting histologically or serologically evident autoimmunities (Fig. 3).

MTLV selectively destroys CD4$^+$ thymocytes/T cells

Inoculation of MTLV on day 0 or 1 led to destruction of thymocytes: total number of thymocytes 2 wk after infection was $5.2 \pm 1.3 \times 10^6$/thymus ($n = 5$), compared with $1.2 \pm 0.1 \times 10^8$/thymus in the mock-infected group ($n = 5$). The depletion was histologically evident in the thymic cortex, and the recovery only left calcifications in the thymus (Fig. 4, *A* and *B*). CD4$^+$8$^+$ thymocytes and CD4$^+$8$^-$ thymocytes were predominantly destroyed in MTLV-infected mice, whereas CD4$^-$8$^+$ thymocytes relatively increased (although the absolute number of CD8$^+$4$^-$ thymocytes decreased in accordance with the reduction in the total number of thymocytes (see above)) (Fig. 4*C*). The thymocyte depletion became detectable by cytofluorometric analysis from 1 wk after inoculation and continued for 1–2 wk; then the number of thymocytes recovered in 1 wk to normal levels and the composition of CD4/CD8 subsets to normal patterns. In the periphery, CD4$^+$ T cells were depleted during the period; the number of CD8$^+$ T cells remained normal (Fig. 4*D*). The total number of spleen cells was not significantly different between MTLV- or mock-infected mice (data not shown), indicating an increase of non-T cells in the infected mice.

MTLV infection on day 7 after birth transiently reduced the number of thymocytes slightly (<20% reduction compared with noninfected mice); the virus infection in young adult (on day 28) did not elicit significant changes in the number or composition of thymocyte/T cell subpopulations (data not shown) in the thymus and periphery, as previously reported by others (19).
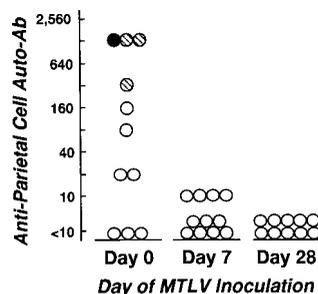

FIGURE 3. Dependency of autoimmune induction on the day of MTLV inoculation. BALB/c mice were inoculated with MTLV on day 0, 7, or 28 after birth. MTLV-inoculated mice were histologically and serologically examined at 3 mo of age. ○, ◍, ●; see legend to Fig. 2.

Enhancement of autoimmune development by Tx

To determine the role of the MTLV-induced T cell deficiency in the autoimmune development, the deficiency was sustained by removal of the thymus 3 wk after neonatal infection, and the mice were examined at 3 mo of age for the development of autoimmune disease (Table I, Fig. 5). The virus-infected and thymectomized (MTLV/Tx) mice developed a higher incidence of histologically evident gastritis compared with virus-infected and sham-Tx mice; the former also developed other autoimmune diseases in a wide spectrum of organs, including the thyroid gland, adrenal glands, salivary glands, and ovaries, but the latter did not. Immunopathology of these autoimmune diseases was similar to those previously reported (18, 33, 34). The control mock infected and thymectomized 3 wk later failed to develop any detectable autoimmunity. In contrast, some of the mice mock infected on day 0 and thymectomized 3 days later developed similar autoimmune gastritis and/or oophoritis. These results indicate that the autoimmunities elicited by neonatal MTLV infection can also be produced by directly manipulating the thymus without virus infection.

Adoptive transfer of autoimmune disease

To confirm the autoimmune nature of the gastric and other lesions in MTLV-infected or MTLV/Tx mice, spleen and lymph node cell suspensions prepared from those BALB/c *nu/+* or *+/+* mice with autoimmune diseases were transferred to BALB/c *nu/nu* mice (Fig. 6). In 2 mo, the transfer of CD4$^+$ cells induced similar histologically evident gastritis accompanying circulating anti-parietal cell autoantibodies. Thyroiditis and oophoritis in MTLV/Tx mice could also be transferred to *nu/nu* mice (data not shown). Thus, self-reactive CD4$^+$ T cells specific for organ-specific Ags appeared to be activated in MTLV-infected or MTLV/Tx mice, mediating the autoimmune diseases.

MTLV was not detected in target organs of autoimmune disease

To determine whether the organs targeted in MTLV-induced autoimmune diseases harbored the virus, the tissue homogenates prepared from various organs of MTLV-infected mice with or without subsequent Tx were inoculated into normal newborn BALB/c mice; the severity of the thymic damage was examined 10 days later (Table II, and see *Materials and Methods*). Irrespective of the severity of the organ-specific autoimmune diseases, the virus was not detected by this bioassay in the affected organs. MTLV was, however, detected in the salivary glands in every assay, although the donor mice showed no inflammation in the gland. This is consistent with the finding by others that MTLV tends to persist in the salivary glands after neonatal infection (20). The virus was not detected in the mice that received mock infection and Tx on day 3
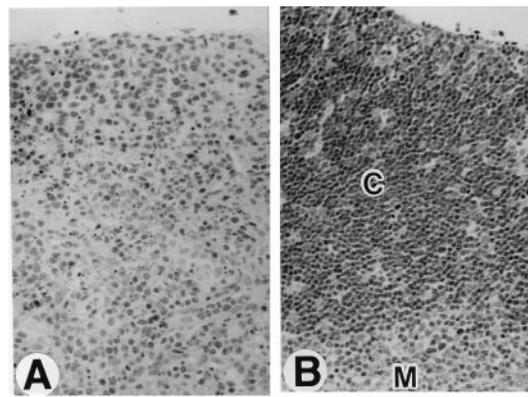
FIGURE 4. Histology of the thymus and composition of T cell subsets after neonatal MTLV infection. *A* and *B*, Histology of the thymus 14 days after neonatal MTLV infection (*A*) or mock infection (*B*) (HE staining, ×100). C and M indicate cortex or medulla, respectively. *C*, Cytofluorometric analysis of thymocyte subpopulations on various days after neonatal MTLV infection. Thymocyte suspensions were stained with FITC anti-CD4 (ordinate) and PE anti-CD8 (abscissa). A representative staining of five independent series of experiments. *D*, Time course of change in T cell subset composition in spleens of neonatally MTLV-infected or mock-infected BALB/c mice. Vertical bars indicate mean ± SD.
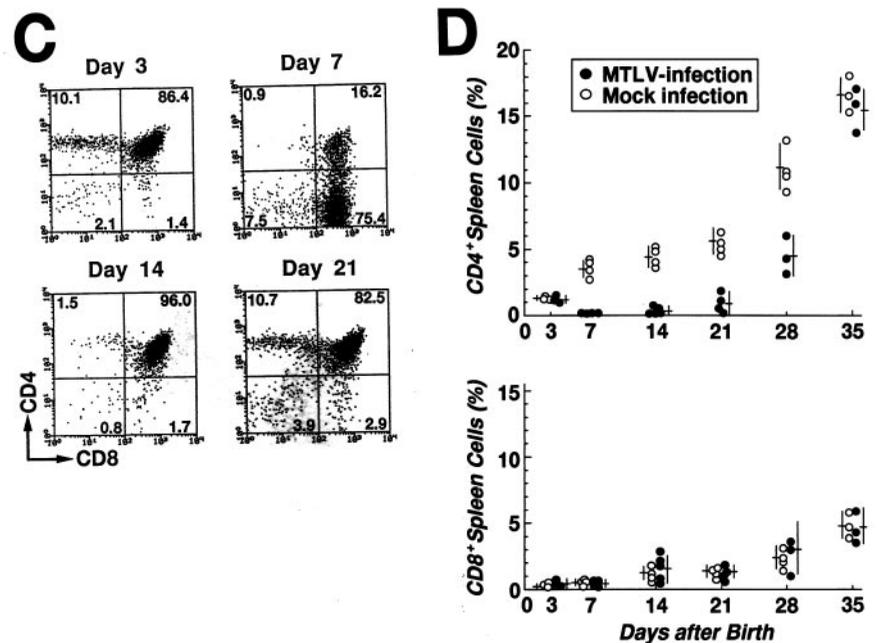
after birth and subsequently developed gastritis, as shown in Fig. 5 (data not shown).

TCR Vβ repertoire in MTLV-infected mice

To assess the possibility that MTLV might be a superantigen and delete, or activate, T cells expressing particular Vβ TCR families, we examined 3-mo-old MTLV/Tx mice with severe autoimmune diseases for the composition of T cell subpopulations expressing particular Vβ domains (Fig. 7). In these mice, peripheral CD4^{+} cells and CD8^{+} cells reduced to nearly one-half their respective numbers in mock-infected and Tx mice, confirming that T cell reduction by MTLV infection (as shown in Fig. 4C) was due to elimination, not sequestration, of T cells. There were no significant differences between the two groups in the percentage compositions of T cells expressing Vβ8.1, 8.2, or Vβ6, or T cells expressing Vβ11, which are normally deleted in BALB/c mice (32) (or T cells

Table I. *Induction of autoimmune disease in BALB/c mice by neonatal MTLV infection and its enhancement by subsequent thymectomy[a]*

Treatment of Mice	No. of mice	No. of mice with autoimmune disease				
		Gastritis	Thyroiditis	Sialoadenitis	Adrenalitis	Oophoritis/orchitis
A. MTLV-infection and Sham-Tx (day 21)	23	7 (30.4)	0	0	0	0
B. MTLV-infection and Tx (day 21)	15	11 (73.3)	1 (6.7)	4 (26.7)	1 (6.7)	3 (20.0)
C. Mock infection	10	0	0	0	0	0
D. Mock infection and Tx (day 21)	15	0	0	0	0	0
E. Mock infection and Tx (day 3)	12	4 (33.3)	0	0	0	3 (25.0)

BALB/c mice were MTLV- or mock-infected on day 0 and thymectomized (Tx) or sham-thymectomized (sham Tx) on day 21; these mice were histologically and serologically examined at 3 mo of age. Mice in group E were mock-infected and thymectomized on day 3. Percent incidences are shown in parentheses. See Fig. 5 for severities of gastritis and titers of anti-parietal cell autoantibodies. See *Materials and Methods* and ref. 33 for histological grading of severity of autoimmune diseases. In group B, one case of thyroiditis was grade 1; two mice developed oophoritis (both grade 2), and one mouse orchitis (grade 1).
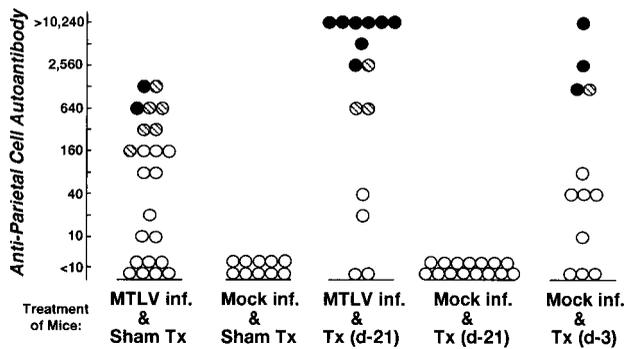
FIGURE 5. Titers of anti-parietal cell autoantibodies in MTLV-infected and subsequently Tx BALB/c mice or mock-infected controls. A group of mice was neonatally mock infected and thymectomized on day 3 after birth. ○, ◎, ●; see legend to Fig. 2.

expressing several other Vβs, such as Vβ3, Vβ5, or Vβ8.3 (data not shown)), among the lymph node CD4$^+$ or CD8$^+$ T cells. Thus, we could not detect a significant alteration of Vβ repertoire, as reported by others for similar autoimmune diseases in mice (35).

Prevention of autoimmune disease by inoculating normal CD4$^+$ T cells after MTLV infection

To determine further the role of the MTLV-induced T cell deficiency in autoimmune development, we inoculated whole, CD4$^+$, or CD8$^+$ splenic T cell suspensions (2×10^7) from normal adult BALB/c mice into MTLV-infected BALB/c mice 3 wk after neonatal virus inoculation, and examined 3 mo later whether the autoimmune development could be prevented (Fig. 8). Inoculation of the whole or CD4$^+$ T cells was effective for prevention, but the same dose of CD8$^+$ cells was not.

Given the recent findings that subpopulations of peripheral CD4$^+$ cells (such as CD5^{high}, CD45RB^{low}, or CD25$^+$CD4$^+$ population) could prevent the development of autoimmune diseases including autoimmune gastritis in day 3 thymectomized mice (see above) (18, 25, 36–40), we examined in MTLV-infected mice whether the autoimmune-preventive activity of normal CD4$^+$ T cells was in CD25$^+$ T cells, which constituted 5–10% of peripheral CD4$^+$ cells and less than 1% of CD8$^+$ cells in normal BALB/c mice (25, 36, 40). Fig. 8 shows that CD4$^+$ cell inocula depleted of
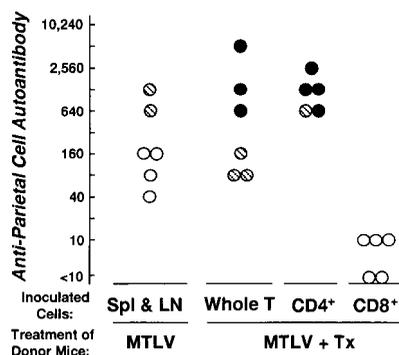

FIGURE 6. Adoptive transfer of autoimmune gastritis to BALB/c *nu/nu* mice. Spleen and lymph node cell suspensions (5×10^7) from 3-mo-old, neonatally MTLV-infected mice, or whole, CD4$^+$, or CD8$^+$ T cell suspensions (2×10^7) prepared from MTLV-infected and subsequently thymectomized mice (see Table I) were i.v. inoculated into 6-wk-old BALB/c *nu/nu* mice, which were histologically and serologically examined 2 mo later. ○, ◎, ●; see legend to Fig. 2.

CD25$^+$ cells failed to prevent the autoimmune development, indicating that CD25$^+$4$^+$ T cells indeed bear the preventive activity in MTLV-induced autoimmunity.

Discussion

We have shown in normal mice that neonatal infection of a CD4$^+$ T cell-tropic virus produces histologically and serologically evident autoimmune diseases, in which CD4$^+$ self-reactive T cells play a key effector role. The autoimmune diseases thus induced are immunopathologically similar to organ-specific autoimmune diseases in humans (41, 42).

A critical question in this virus-induced autoimmunity is then whether the virus affected the organs/tissues to be targeted by the autoimmune responses, thereby leading to activation of self-reactive T cells (e.g., by changing antigenicity of self molecules, aberrantly presenting self peptides to T cells, bystander activation of self-reactive T cells in the virus-infected tissues, or molecular mimicry of self constituents); alternatively, it primarily affected the immune system, leading to altered immunologic control of self-reactive T cells. The latter appears to be the case for the following reasons. First, MTLV was not detected by bioassay in the organs/tissues (such as the gastric mucosa) targeted in the autoimmune disease; on the other hand, little pathologic alteration was observed in the salivary glands, where the virus persistently infected. Second, inoculation of MTLV into 1-wk-old or adult mice, even more than once at large doses, was unable to elicit the autoimmunity, albeit the virus became persistently infected in the salivary gland. These results indicate that, even if the virus persistently infected the gastric mucosa at the level undetectable by our bioassay, the infection per se might be unable to cause autoimmune disease. Third and most importantly, a similar spectrum of autoimmune diseases with similar immunopathology can be produced in normal mice even in the germfree condition by simply manipulating the thymus/T cells (e.g., neonatal thymectomy as shown in Table I and Fig. 5); and such autoimmune development can be prevented by inoculating normal CD4$^+$ T cells, especially CD25$^+$4$^+$ T cells (18, 25, 36–40, 43–45). Sialoadenitis as observed in MTLV/Tx mice can develop as well without virus infection of the salivary glands (33, 34, 36). Thus, although MTLV may not be a superantigen deleting thymocytes expressing a particular TCR Vβ (Fig. 7), it may affect the thymic clonal deletion mechanism, leading to enhanced production of the pathogenic self-reactive T cells, which might easily expand in a T cell-depleted periphery of MTLV-infected neonates (17). Alternatively, although not mutually exclusive, MTLV may deplete/reduce the immunoregulatory CD4$^+$ thymocytes/T cells for a limited period, meanwhile allowing certain self-reactive CD4$^+$ T cells to become activated and expand (see below) (34, 44).

We have shown previously that CD25$^+$4$^+$ T cells with autoimmune preventive activity are produced continuously by the normal thymus (59), and ontogenically begin to migrate to the periphery at about day 3 after birth in normal mice (25); transient elimination of peripheral CD25$^+$4$^+$ T cells by Tx at about day 3 after birth (Table I, Fig. 5), or direct elimination from adults by anti-CD25 Ab, produced various autoimmune diseases including autoimmune gastritis in normal BALB/c mice; and reconstitution of normal CD25$^+$4$^+$ T cells prevented the autoimmune development (25, 36). In the present experiments, inoculation of CD4$^+$ cells from syngeneic noninfected adult mice prevented the autoimmune development in neonatally MTLV-infected mice, but the same dose of CD25$^-$4$^+$ T cells or CD8$^+$ T cells from the same donors did not (Fig. 8). Furthermore, relatively few CD25$^+$4$^+$ T cells, compared with the number of CD25$^-$4$^+$ cells, were present in the periphery

Table II. *Biological assay of MTLV in various organs of neonatally infected BALB/c mice with autoimmune disease[a]*

Mouse No.	Treatment of Donor Mouse	Autoimmune Disease in Donor Mouse	Detection of MTLV in			
			Stomach	Ovaries	Testes	Salivary glands
1	MTLV	Gastritis (1^0)[b]	0/14[c]	ND	ND	2/2
2	MTLV		ND	ND	ND	6/8
3	MTLV+Tx	Gastritis (2^0)/Orchitis (1^0)	0/9	ND	0/12	5/8
4	MTLV+Tx	Gastritis (2^0)	0/9	0/8	ND	12/14
5	MTLV+Tx	Gastritis (2^0)	0/8	ND	ND	ND
6	MTLV+Tx		0/16	0/12	ND	7/10

[a] Homogenates of organs/tissues of 3-mo old BALB/c mice MTLV-infected on day 0, or MTLV-infected and subsequently thymectomized (Tx) (as shown in Table 1 and Fig. 5), were i.p. inoculated into newborn BALB/c mice, which were histologically examined for necrosis of the thymus 10 days later. As positive or negative control, thymus homogenates prepared for the passage of MTLV or those prepared from normal noninfected BALB/c mice, respectively, were inoculated. The former inocula caused macroscopically and histologically evident thymic damage in all mice, whereas the latter produced no damage in all mice.

[b] 1^0, 2^0; grade 1 and 2, respectively, of histological severity of autoimmune disease (see ref. 33 for histological grading).

[c] Number of newborn mice with thymic necrosis among total newborn mice inoculated with homogenates from each organ. ND, not determined.

in the initial recovering phase after neonatal MTLV infection (our unpublished data), although the time course of T cell recovery was variable among individual mice (Fig. 4C). These results, when taken together, suggest that MTLV might affect the CD25$^+$4$^+$ T cell-mediated control of self-reactive T cells (e.g., by reducing immunoregulatory CD25$^+$4$^+$ thymocytes/T cells, as in neonatal Tx), thereby leading to the development of autoimmune disease, and that the enhancement of autoimmune development by Tx subsequent to neonatal MTLV infection could be attributed to sustained deficiency of the immunoregulatory CD25$^+$4$^+$ T cells. This possibility is currently under investigation.

The assumption that MTLV may affect the immune system, not the target self Ags, poses the question as to how particular self Ags, such as the gastric parietal cell Ag, are selectively aggressed

by the MTLV-induced autoimmune responses. The difference in the incidence of histologically evident gastritis between BALB/c and DBA/2, which share *d* haplotype of MHC, or between A and C3H, which share *k* haplotype of class II MHC, indicates that host genetic factors, including non-MHC genes, significantly contribute to determining the susceptibility to the autoimmunity, especially to the development of histologically evident autoimmune disease (Fig. 2). Although each strain might be different in the susceptibility to MTLV infection itself, it is of note that the strain differences in the susceptibility/resistance to various autoimmune diseases in neonatally MTLV-infected mice were similar to those observed in autoimmune induction by other ways of manipulating the thymus/T cells. For example, BALB/c predominantly developed autoimmune gastritis when the thymus/T cells were affected by a physical or chemical agent (34, 44) or by a genetic manipulation (33), whereas other strains predominantly developed other autoimmune diseases or no autoimmune disease. These findings collectively indicate that the host genetic elements may be mainly responsible for determining the phenotype of autoimmune disease (i.e., which self-reactive T cell clones are more prone to be activated) upon introduction of abnormal control of self-reactive T cells by MTLV infection. Current efforts by us and others to map these genes on chromosomes have revealed significant contributions of both MHC and non-MHC genes of the hosts to determining the autoimmune phenotype (Fig. 2) (33, 34, 44–46) (N. Sakaguchi et al., manuscript in preparation).
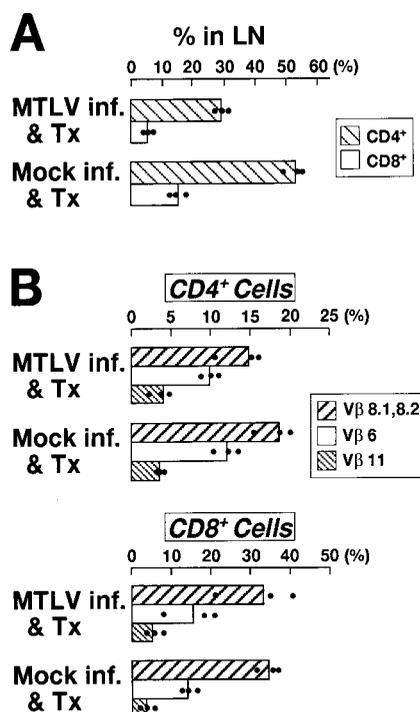
FIGURE 7. Repertoire of T cells expressing particular Vβ domains in MTLV/Tx BALB/c mice. Lymph node cell suspensions from MTLV/Tx BALB/c mice (*n* = 3), or control mock-infected and Tx mice (*n* = 3), at 3 mo of age were analyzed for percentage composition of CD4$^+$ or CD8$^+$ T cells (*A*), or composition of T cells expressing particular Vβ domains among CD4$^+$ or CD8$^+$ cells (*B*).
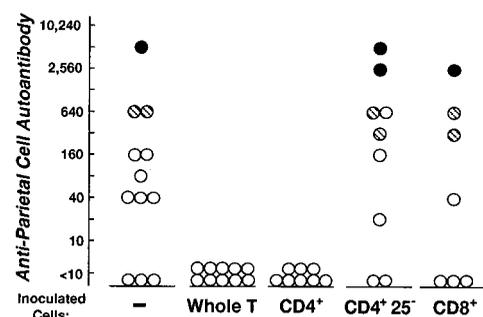
FIGURE 8. Prevention of autoimmune disease in neonatally MTLV-infected mice by inoculating CD4$^+$ cells from syngeneic noninfected adult mice. BALB/c mice inoculated with MTLV on day 0 were inoculated with whole, CD4$^+$, or CD8$^+$ splenic T cells (2 × 10^7) from 3-mo-old normal BALB/c mice on day 21, and histologically and serologically examined at 3 mo of age. Another group of MTLV-infected mice received the same number of CD25$^-$CD4$^+$ T cells prepared by anti-CD25 Ab and complement treatment. ○, ◒, ●; see legend to Fig. 2.

The MTLV-induced autoimmune disease in mice might have a common pathogenetic basis with similar autoimmune diseases that have been reported in other species to be linked with virus infection. In humans, for example, congenital rubella virus infection, which transiently reduces the number of T cells and affects T cell functions (47), resulted in later development of IDDM and other organ-specific autoimmune diseases in genetically susceptible individuals (48–50). A vertically transmitted virus producing granulomatous lesions in the thymus led to the development of systemic as well as organ-specific autoimmune diseases (including autoimmune gastritis and IDDM) in a colony of dogs (51, 52). The Kilham's virus, which does not directly attack the pancreatic β cells, elicited IDDM in a strain of rats genetically susceptible to IDDM and other autoimmunities (including autoimmune gastritis) (53–55). The present findings in MTLV-induced autoimmunity suggest that these viruses may also alter the thymic and/or peripheral control of self-reactive T cells by infecting thymocytes/T cells (especially CD4$^+$ population), not the target self Ags; and certain host genes (some of which might be common among these species as shown for IDDM (56)) may determine the phenotype of the autoimmune disease thus triggered. Our findings further suggest that more than one virus might elicit the same autoimmune disease by similarly affecting the T cell immune system, and, consequently, it might be unnecessary to postulate a specific etiologic agent for each autoimmune disease.

There are many viruses (besides rubella virus) capable of infecting human T cells (2, 12–14), even destroying thymocytes/T cells (57). Some of these T cell-tropic viruses may play an etiologic role in human autoimmune disease through a "hit and run" alteration of the T cell immune system, as shown in murine MTLV infection. A similar mechanism could also be responsible for autoimmunity observed in HIV infection (23, 24), since HIV reportedly first infects and destroys CD25$^+$4$^+$ T cells (58). The MTLV-induced autoimmunity would be a suitable model for studying virus-induced autoimmunity in humans.

Acknowledgments

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