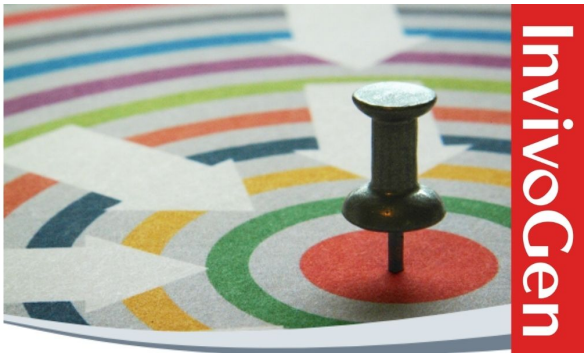

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Ionizing radiation and autoimmunity. Induction of autoimmune disease in mice by high dose fractionated total lymphoid irradiation and its prevention by inoculating normal T cells. FREE

N Sakaguchi; ... et. al

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Ionizing Radiation and Autoimmunity

Induction of Autoimmune Disease in Mice by High Dose Fractionated Total Lymphoid Irradiation and Its Prevention by Inoculating Normal T Cells[1]

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Ionizing radiation can functionally alter the immune system and break self-tolerance. High dose (42.5 Gy), fractionated (2.5 Gy 17 times) total lymphoid irradiation (TLI) on mice caused various organ-specific autoimmune diseases, such as gastritis, thyroiditis, and orchitis, depending on the radiation dosages, the extent of lymphoid irradiation, and the genetic background of the mouse strains. Radiation-induced tissue damage is not the primary cause of the autoimmune disease because irradiation of the target organs alone failed to elicit the autoimmunity and shielding of the organs from irradiation was unable to prevent it. In contrast, irradiation of both the thymus and the peripheral lymphoid organs/tissues was required for efficient induction of autoimmune disease by TLI. TLI eliminated the majority of mature thymocytes and the peripheral T cells for 1 mo, and inoculation of spleen cell, thymocyte, or bone marrow cell suspensions (prepared from syngeneic nonirradiated mice) within 2 wk after TLI effectively prevented the autoimmune development. Depletion of T cells from the inocula abrogated the preventive activity. $CD4^+$ T cells mediated the autoimmune prevention but $CD8^+$ T cells did not. $CD4^+$ T cells also appeared to mediate the TLI-induced autoimmune disease because $CD4^+$ T cells from disease-bearing TLI mice adoptively transferred the autoimmune disease to syngeneic naive mice. Taken together, these results indicate that high dose, fractionated ionizing radiation on the lymphoid organs/tissues can cause autoimmune disease by affecting the T cell immune system, rather than the target self-Ags, presumably by altering T cell-dependent control of self-reactive T cells. *Journal of Immunology,* 1994, 152: 2586.

T cells are key mediators of many organ-specific autoimmune diseases, such as autoimmune thyroiditis, gastritis, and insulitis in insulin-dependent diabetes mellitus (1–6). To maintain immunologic self-tolerance and prevent autoimmune disease, the pathogenic self-reactive T cells must be eliminated in the thymus or, when produced by the thymus, their expansion/activation must be controlled in the periphery (7–10). Autoimmune disease may develop when exogenous insults affect the thymus and elicit the production of the pathogenic self-reactive T cells and/or prepare the immunologic conditions favorable to their peripheral expansion/activation (11, 12). Here, we show that ionizing radiation has such an autoimmune-inducing effect.

Biologic effects of ionizing radiation depend on the character of the radiation, the total dose administered, the time when the dose is given, and the site of irradiation (13, 14). High dose irradiation can be achieved by low dose fractionation and by shielding vital organs. One such radiation protocol, called TLI,[3] consists of irradiating major lymphoid organs, including the thymus and the spleen, while shielding radiosensitive nonlymphoid organs (e.g., long bones, lung, and skull) with lead plates (15, 16). Total dose of 30 to 40 Gy is achieved within 4 to 5 wk by

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[3] Abbreviations used in this paper: TLI, total lymphoid irradiation; Tx, thymectomy; H&E, hematoxylin and eosin; BM, bone marrow; GvHD, graft-versus-host disease; CsA, cyclosporin A.

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multiple small fractions of 2 to 2.5 Gy each. TLI has been routinely used for treatment of lymphoid malignancies in humans for the past 30 yr (15). It is an effective treatment for various autoimmune diseases in humans and rodents (17–21). Furthermore, a unique immunologic effect of TLI is that it establishes allograft tolerance in humans and animals when allogeneic bone marrow cells or solid organs are engrafted immediately after TLI (22–26).

In this report, we show that TLI paradoxically breaks self-tolerance in selected strains of normal mice and elicits T cell-mediated organ-specific autoimmune diseases by affecting the T cell immune system not the target self-Ags. Furthermore, the development of autoimmune disease can be prevented by inoculating T cells from syngeneic nonirradiated mice. This is an example of autoimmune disease caused by physical insult to the immune system and would contribute to our understanding of the cause and mechanism of autoimmune disease in humans.

Materials and Methods

Mice

BALB/cKa, C57BL/Ka, and C3H/Km mice were obtained from the specific pathogen-free colony of Dr. Robert Kallman, Department of Radiology, Stanford University School of Medicine. Male BALB/c *nu*/+ mice and 6-wk-old male athymic BALB/c-*nu*/*nu* mice were purchased from Life Science Associates, St. Petersburg, FL. SWR/J and A/J mice were purchased from the Jackson Laboratory, Bar Harbor, ME. (C57BL/6J × BALB/cByJ)F_1 (B6CF1) and DBA/2J mice were provided from the vivarium of the Scripps Research Institute.

TLI

Male mice of 3 to 4 mo of age were used for TLI. Mice were anesthesized with Nembutal (pentobarbital; Abbott Laboratories, Chicago, IL) and positioned in an apparatus designed to irradiate the major lymphoid organs, including the spleen, thymus, and cervical, axillary, mediastinal, inguinal, and mesenteric lymph nodes (22, 23). The skull, lungs, ribs, thoratic spines, tail, and hind legs were shielded with lead. The mice were given 2.5 Gy/day, 5 times/wk to a total accumulative dose of 42.5 Gy over 17 treatments. Irradiation was delivered from a single 250 kV (15A) source (Phillips Medical System Inc., Shelton, CT). The dose was 0.93 Gy/min using a 0.35-mm Cu filter and a 52-cm source-axis distance. A group of mice was irradiated at the Scripps Research Institute from a ^{137}Cs source (Gammacell 40 irradiator; Atomic Energy of Canada, Ottawa, Canada) at 0.813 Gy/min with extra lead plates for shielding of vital organs. Neomycin (Pharma-Tek, Inc., Huntington, NY) and polymyxin B (Sigma, St. Louis, MO) were added to the drinking water during TLI and for 1 mo after completion of TLI. For partial irradiation of lymphoid organs, a part of the body was shielded by an additional lead plate.

ELISA

Details of the ELISA (using alkaline phosphatase-conjugated secondary Ab and *p*-nitrophenyl disodium hexahydrate as the substrate) for detecting Abs against the gastric parietal cells, thyroglobulins, ss or dsDNA, or trinitrophenyl haptens and the ELISA for measuring serum immune complexes or IgG concentration were previously described (12, 27). Titers of autoantibodies against the parietal cells or thyroglobulins were defined as the highest dilution at which the absorbance at 405 nm is over 0.1 or 0.05, respectively, when test sera are serially twofold diluted from 1/10 dilution, because the absorbance at 1/10 dilution of 3- to 4-mo-old normal BALB/c mice was 0.07 ± 0.028 (n = 50) in the antiparietal cell ELISA and 0.03 ± 0.012 (n = 20) in the antithyroglobulin ELISA (27).

Immunohistology

Stomachs were embedded in OCT compound (Miles Inc., Chicago, IL) and instantaneously frozen in liquid nitrogen. Cryostat tissue sections were incubated with monoclonal antiparietal cell Ab (IgG2b) that was prepared from a mouse with autoimmune gastritis induced by neonatal CsA treatment (27, and manuscript in preparation). After washing with PBS, the slides were incubated with peroxidase-conjugated goat anti-mouse IgG (Caltag Laboratories, San Francisco, CA) then washed and incubated with diaminobenzidin and H_2O_2. The slides were then counterstained with hematoxylin.

Cell preparation

Spleen cell suspensions (4×10^7) were treated with anti-Thy-1.2 (mouse IgM) (28), anti-L3T4 (rat IgG2b) (29), or anti-Lyt-2.2 (mouse IgG2a) (30) and nontoxic rabbit C, as previously described (11), and i.v. inoculated into mice. The hybridoma cell lines secreting these mAbs were obtained from American Tissue Culture Collection, Rockville, MD.

Cytofluorometric analysis of T cell subsets

FITC-labeled anti-Thy-1.2 and anti-Lyt-2 mAbs and phycoerthrin-labeled anti-L3T4 mAbs were purchased from Becton Dickinson, San Jose, CA. Biotinylated mAbs specific for TCR Vβ11 (RR 3-15) or Vβ6 domain (RR 4-7) were provided by Dr. O. Kanagawa, Washington University, St. Louis, MO (31, 32). Anti-Vβ3 (KJ23) (33) and anti-Vβ8.1 and 8.2 Abs (MR5-2) (34) were purchased from PharMingen, San Diego, CA. Staining procedure was as previously described (27). Stained cells were analyzed by an FACScan or FACS II flow cytometer (Becton Dickinson).

Other methods

When mice were killed, tissues (thyroids, salivary glands, lungs, stomach, pancreas, adrenal glands, liver, kidneys, ovaries, or testes) were fixed in 10% formalin and processed for histologic sectioning and staining with H&E (11, 27).

Results

Male BALB/c mice at 3 mo of age were used in the following experiments unless indicated otherwise.

Development of organ-specific autoimmune disease, especially gastritis, in BALB/c mice after TLI

A total of 55 BALB/c mice were given TLI in the various experiments described below. They were allowed to survive for 6 mo and examined for serologic and/or histologic occurrence of autoimmunity (Table I). Seventy-five percent of the TLI mice developed histologically evident gastritis. Approximately 60% showed achlorhydria (pH 7 to 8 of the gastric contents compared with pH 3 to 4 of control nonirradiated mice) and macroscopically evident giant rugae formation due to destruction of the gastric parietal cells and compensatory hyperplasia of mucous cells (see below). The majority (~90%) developed antiparietal cell autoantibodies (assessed by ELISA) in the circulation (see refs. 11 and 27 for macroscopic view of the giant rugae and antiparietal cell autoantibody demonstrated by indirect immunofluorescence staining).

Autoantibodies against gastric parietal cells reacted with a ~90 kDa gastric membrane component, similar to the H^+-K^+-ATPase reported for human autoimmune gastritis/pernicious anemia (6) by Western blotting technique (S. Sakaguchi and N. Sakaguchi, unpublished data). Two

Table I. *Incidence of autoimmune disease in BALB/c male mice given TLI*

Gastritis	Thyroiditis	Orchitis/ Epididymitis	Sialoadenitis
41/55[a] (74.5) (2°; 35 1°; 6)[b]	2/55 (3.6) (1°; 2)	5/55 (9.1) (2°; 2 1°; 3)	3/55 (7.3)

[a] Three-month-old male BALB/c mice were given TLI. Incidence of histologically evident autoimmune disease, assessed 5 to 6 mo after TLI, is shown with percentage incidence in parentheses. Thirty-five (63.6%) mice showed achlorhydria (pH 6–8 of gastric contents compared with pH 3–4 of control nonirradiated mice) and macroscopically evident giant rugae formation due to severe destruction of gastric parietal cells and compensatory hyperplasia of mucous cells (grade 2) (see Fig. 3*C* and ref. 11 for macroscopic view of giant rugae). In six (10.9%) mice, gastritis was histologically observed but not macroscopically evident (grade 1) (see Fig. 3*B*). Two cases of thyroiditis were histologically evident but showed no goiter formation (grade 1) (11). In two cases of orchitis, both testicles and epidydimes were destroyed and aspermatogenic (grade 2); in three cases, epidydimitis was histologically evident but orchitis was mild (grade 1) (see ref. 11 for histologic details).

[b] 1°, grade 1; 2°, grade 2.

(3.6%) mice histologically developed thyroiditis with serum antithyroglobulin titer of 320 and 640 by ELISA, respectively; ~20% had low but significant titers of antithyroglobulin autoantibody (10 to 40 by ELISA) without histologically evident thyroiditis. Approximately 10% developed epidydimitis and/or orchitis; ~7% showed infiltration of inflammatory cells in the salivary gland. Other organs were histologically normal.

Time course of autoimmune development and correlation between histologic severity and autoantibody titer

The antiparietal cell autoantibodies became detectable by ELISA from 1 mo after TLI; the titers steadily rose in approximately 2 mo and stayed constant for the rest of the observation period (Fig. 1). The grade of histologic severity of the gastritis was well correlated with the titer of antiparietal cell autoantibody.

Dose responses

Mice given 4, 8, or 17 TLI treatments were examined 6 mo later for the degree of histologic and serologic gastric autoimmunity (Fig. 2). Histologically evident gastritis developed only in the group given 17 TLI treatments. The eight TLI treatments induced low titers of antiparietal cell autoantibodies in 20% of mice but apparently no histologic changes in the gastric mucosa.

Immunopathology of autoimmune disease

Damage to the gastric parietal cells and infiltration of inflammatory cells became histologically evident 4 to 6 wk after completion of TLI (Fig. 3*A*). The lesions had progressed in 6 mo to severe infiltration of mononuclear cells, complete loss of parietal cells and chief cells, and hyperplasia of mucous cells (Fig. 3, *B* and *C*). Immunohistochemical staining of the gastric mucosa with antiparietal cell mAbs showed no pathologic changes in the parietal cells 1 wk after completion of TLI (Fig. 3*D*); in contrast, the parietal cells were lost or destroyed in the gastric mucosa 6 mo after TLI (Fig. 3*E*). The class II MHC Ags were expressed on the gastric epithelium afflicted with histologically evident gastritis but not on the apparently intact

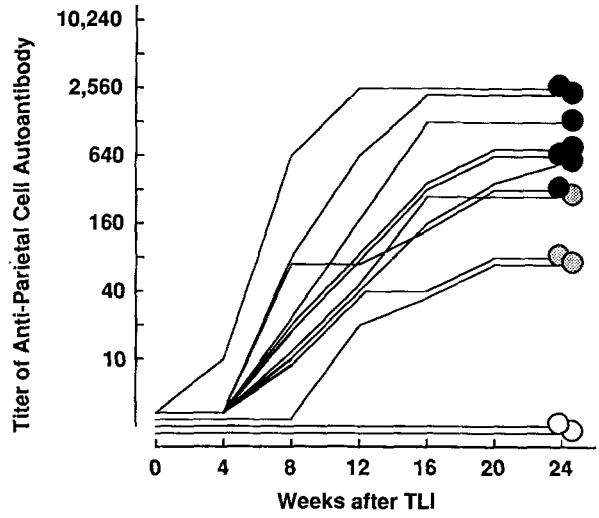

FIGURE 1. Time course of development of gastric autoimmunity after TLI. Male BALB/c mice (3 mo old) were given TLI, checked every 4 wk for serum titers of antiparietal cell autoantibodies by ELISA, and examined for histologic occurrence of gastritis 6 mo after completion of TLI. ○, Histologically intact gastric mucosa; ●, gastritis histologically evident with destruction of parietal cells and cellular infiltration into gastric mucosa as shown in Figure 3*B* (grade 1); ●, severe destruction of gastric mucosa with formation of giant rugae due to compensatory hyperplasia of mucous cells as shown in Figure 3*C* (grade 2) (see ref. 11 for giant rugae).

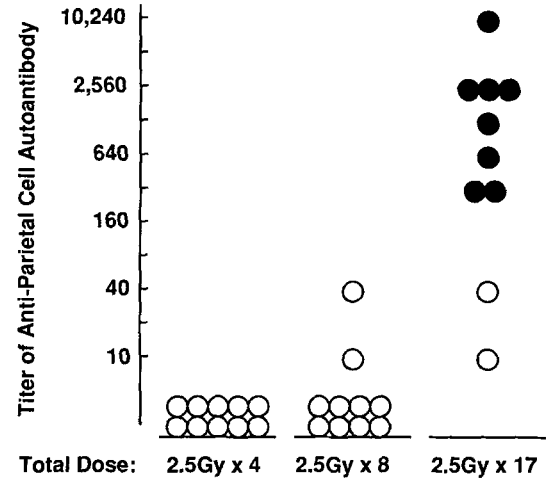

FIGURE 2. Dose effects of TLI on development of gastric autoimmunity. Male BALB/c mice (3 mo old) were given 4, 8, or 17 times of TLI and examined for histology of stomach and titer of antiparietal cell autoantibodies 6 mo after completion of TLI. ○, ●; See Figure 1.

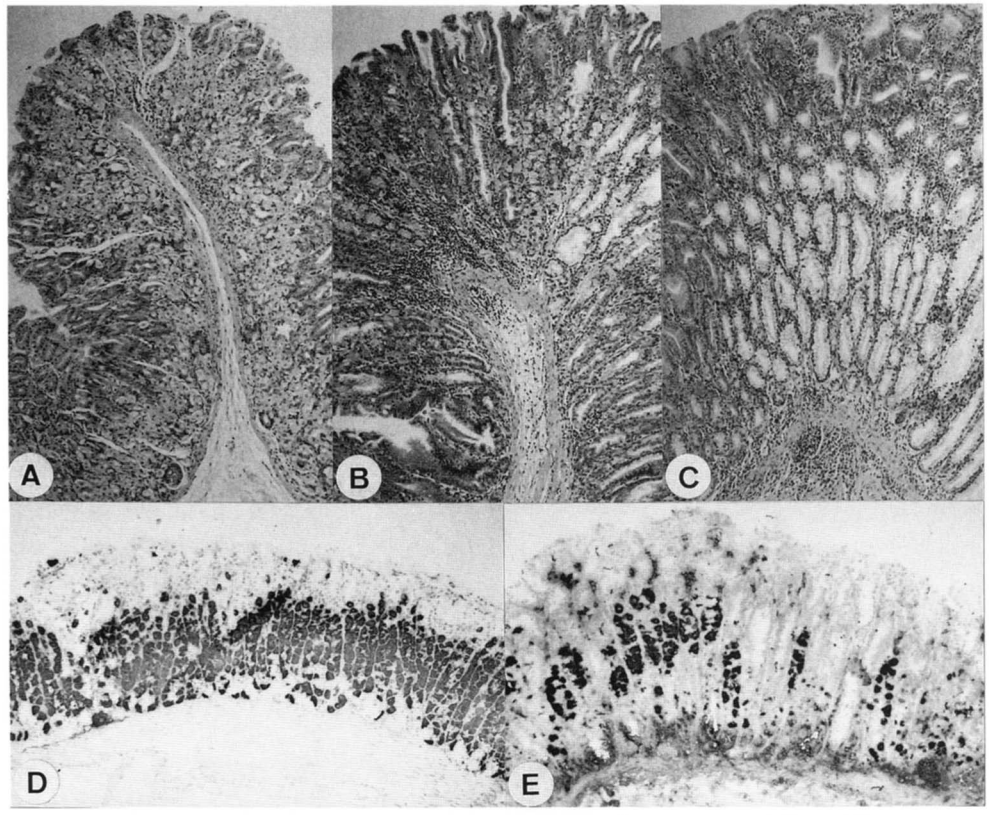

FIGURE 3. Immunopathology of autoimmune gastritis. Gastric mucosa of mice 1 mo (*A*), 2 mo (*B*), or 6 mo (*C*) after TLI (H&E staining). Gastritis progressed from mild infiltration of mononuclear cells (*A*) to severe infiltration and damage of parietal cells (grade 1) (*B*), and to complete loss of parietal cells and chief cells with replacement by mucous cells (grade 2) (*C*). Immunoperoxidase staining with parietal cell-specific mAbs shows intact parietal cells in gastric mucosa 1 wk after completion of TLI (*D*) but loss and damage of parietal cells in gastric mucosa 6 mo after TLI (*E*). *A* to *E*; ×100.

epithelium immediately after TLI (data not shown). Immunopathology of thyroiditis, orchitis/epididymitis, and sialoadenitis was similar to that described elsewhere (11, 27).

Adoptive transfer of autoimmune disease by T cells

TLI was given to 12 BALB/c *nu*/+ mice (3 mo of age) whose testes were carefully shielded by additional lead plates during TLI. Nine (75%) mice developed histologically severe gastritis 4 mo after TLI; two of them also suffered from epidydimitis and low grade orchitis (see ref. 11 for immunopathology of epididymitis/orchitis). Spleen and lymph node cell suspensions (4×10^7) prepared from individual mice were i.v. inoculated into 6-wk-old syngeneic BALB/c *nu*/*nu* mice; the *nu*/*nu* mice were histologically and serologically examined 2 mo later.

Table II shows that the lymphocytes could adoptively transfer the autoimmune disease, and removal of $CD4^+$ ($L3T4^+$) cells eliminated the transfer activity of the lymphocytes, whereas removal of $CD8^+$ ($Lyt\text{-}2^+$) cells did not. The result indicates that $CD4^+$ cells can transfer the disease in a disease-specific manner by helping B cells to form organ-specific autoantibodies and, presumably, by eliciting cell-mediated immune responses to the target self-Ags (35). In these transfers, however, approximately 50% of the recipient *nu*/*nu* mice (10 of 22 mice), especially those transferred with lymphocytes treated with C

Table II. *Adoptive transfer of autoimmune disease to syngeneic nu/nu mice by $CD4^+$ T cells*

Autoimmune Disease in Donor TLI Mice[a]	Treatment of Lymphocytes[b]	Autoimmune Disease in Recipient *nu/nu* Mice[c]	
		Gastritis	Orchitis
	C	3/3 (640, 320, 320)	0/3
Gastritis (7)	Anti-Thy-1.2+C	0/2 (10, <10)	0/2
	Anti-Lyt-2.2+C	3/3 (640, 640, 320)	0/3
	Anti-L3T4+C	0/3 (10, <10, <10)	0/3
Gastritis and	C	1/1 (320)	1/1
Orchitis (2)	Anti-Thy-1.2+C	0/1 (<10)	0/1

[a] Male BALB/c *nu*/+ mice (3 mo old) were given TLI with careful shielding of testes with extra lead plate. Spleen and lymph node cell suspensions prepared from the *nu*/+ mice with respective autoimmune disease(s) at 4 mo after TLI were i.v. transferred to 6- to 8-wk-old BALB/c *nu*/*nu* mice. Number of mice used as donors is shown in parentheses.

[b] Spleen and lymph node cell suspensions (4×10^7) were treated with antisera and rabbit complement (C) before transfer.

[c] Incidence of histologically evident autoimmune diseases in *nu*/*nu* mice that survived 2 mo after cell transfer. Gastritis in *nu*/*nu* recipients was all grade 2. Titers of antiparietal cell autoantibodies assessed by ELISA for individual *nu*/*nu* mice are shown in parentheses.

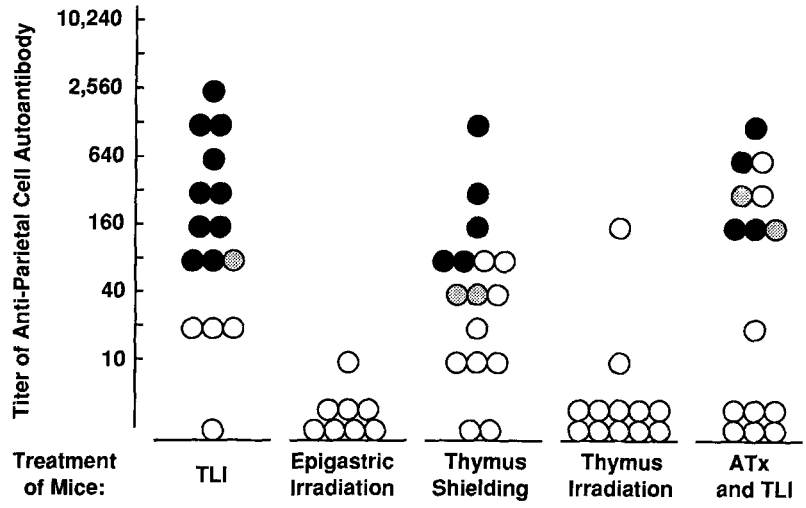

FIGURE 4. Effects of epigastric irradiation, thymic irradiation, thymus shielding, or adult thymectomy (ATx) on induction of autoimmune disease by TLI. Male BALB/c mice (3 mo old) were irradiated 17 times with 2.5 Gy each on epigastric region or on thymus and neck region with shielding of other body parts, or given TLI with shielding of thymus. A group of mice received thymectomy at 2 mo of age and TLI 1 mo later. These mice were examined 6 mo later for histologic and serologic development of autoimmune gastritis. ○, ●, ●; See Figure 1.

only (6 of 9 mice) or with anti-CD8 and C (3 of 7 mice), succumbed to wasting disease and died within 2 wk after cell inoculation. The wasting disease was clinically similar to GvHD but there appeared to be no significant histoincompatibility between the *nu/nu* mice and the *nu/+* mice used in these experiments because transfer of spleen cell suspensions from nonirradiated BALB/c *nu/+* mice to BALB/c *nu/nu* mice did not elicit the GvHD (data not shown).

Effects of partial irradiation on induction of autoimmunity

To determine whether the TLI-induced gastritis was due to direct radiation damage to the gastric mucosa, the fundal region of the stomach was irradiated 17 times at 2.5 Gy per irradiation with shielding of other organs including the spleen (Fig. 4). Serologically or histologically evident gastric autoimmunity did not develop in these mice, suggesting that possible radiation damage to the gastric mucosa per se is unable to elicit the autoimmunity. To determine the role of the thymus in this T cell-mediated autoimmunity, TLI was given either with shielding of the thymus or only to the thymus and neck region by shielding the rest of the body. The thymus shielding significantly reduced the histologic incidence of gastritis (43.8 vs 73.3%, $0.025 < p < 0.05$ by χ^2 test). The thymus/neck irradiation alone (by shielding the abdomen) failed to elicit the autoimmunity. Thus, irradiation of both the thymus and peripheral lymphoid organs/tissues appeared to be required for efficient induction of autoimmune disease by TLI.

Effects of thymectomy before TLI on induction of autoimmune disease

When 2-mo-old BALB/c mice were thymectomized and given TLI 1 mo later, they developed gastritis, indicating that autoimmune T cells could develop from the peripheral T cell pool. However, the development of autoantibodies

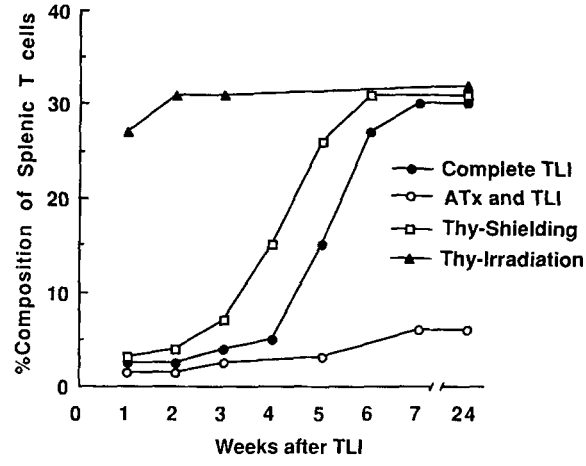

FIGURE 5. Recovery of T cells after TLI. Percentage of Thy-1$^+$ cells in spleens of four groups of mice shown in Figure 4 was assessed at various time points after irradiation. Each point represents average value of two mice.

in these mice took more time, and the histologic incidence of gastritis was significantly lower than TLI on nonthymectomized mice (40 vs 73.3%, $0.05 < p < 0.1$ by χ^2 test) (Fig. 4).

Recovery of T cells after complete or partial TLI

TLI depleted both CD4$^+$ cells and CD8$^+$ cells from the periphery. It took 6 to 8 wk for the number of T cells and composition of T cell subsets to recover to the control level after completion of TLI (Fig. 5). The T cell recovery was faster in the group with thymus shielding. Thymus and neck irradiation alone did not significantly change the number or composition of splenic T cells. The percentage of splenic T cells in the Tx-TLI mice recovered slowly over 6 mo to only 5 to 10% of the total spleen cells.

Several reports have suggested that changes in the composition of T cells expressing a particular TCR Vβ domain

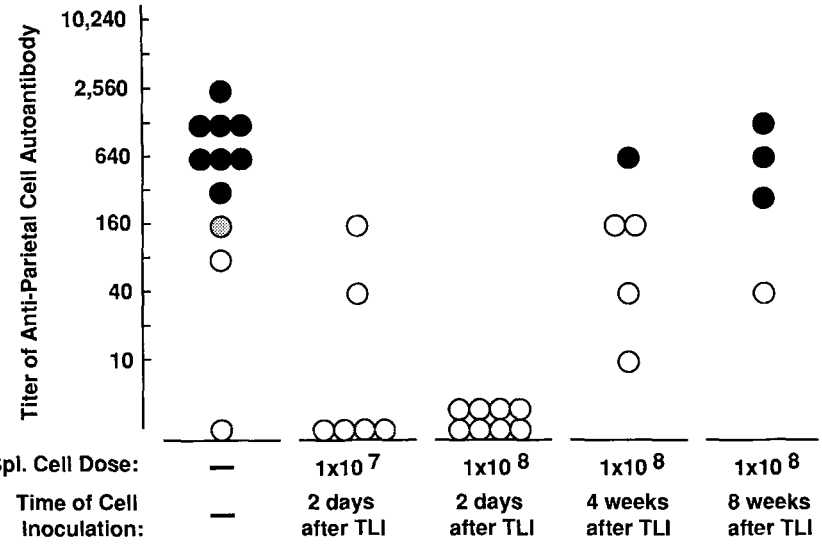

FIGURE 6. Prevention of autoimmune disease by inoculating spleen cells from syngeneic nonirradiated mice. Male BALB/c mice (3 mo old) received TLI and inoculation of normal BALB/c spleen cell suspensions (1×10^7 or 1×10^8) 2 days, 4 wk, or 8 wk after completion of TLI. The mice were histologically and serologically examined 6 mo after TLI. ○, ●, ●; See Figure 1.

might contribute to development of organ-specific autoimmunities; e.g., $V\beta11^+$ cells, the majority of which are deleted in normal BALB/c mice, were found to increase in neonatally thymectomized BALB/c mice, some of which spontaneously developed autoimmune gastritis similar to the one after TLI (36–39). Compared with the post-thymectomy autoimmune model, there was not a marked difference between TLI BALB/c mice 3 mo after TLI and age-matched control BALB/c mice in percentage of composition of lymph node α/β TCR^+ cells expressing the following TCR Vβ domains: Vβ3 (0.09 ± 0.05 in TLI mice vs 0.06 ± 0.05 in control mice); Vβ8.1, 8.2 (23.36 ± 1.23 vs 25.91 ± 1.34); Vβ11 (2.04 ± 0.96 vs 1.73 ± 0.50) (the mean ± SD, $n = 3$).

Prevention of autoimmune disease by inoculating lymphocytes from syngeneic nonirradiated mice

To determine whether restoration of the TLI-induced lymphocyte depletion can prevent or ameliorate autoimmune disease, spleen cell suspensions (1×10^7 or 1×10^8) prepared from syngeneic nonirradiated mice were i.v. inoculated (Fig. 6). The inoculation of 1×10^7 cells 2 days after TLI completely prevented the occurrence of gastritis; however, the cell inoculation 4 or 8 wk after TLI was less effective and ineffective, respectively, even with the dose of 1×10^8 cells.

Inoculation of thymocytes (1×10^8) or BM cells (3×10^7) immediately after TLI significantly reduced the incidence/severity of gastritis, although less effectively than the spleen cell inoculation (Fig. 7). Depletion of T cells from the splenic or BM cell inocula abrogated the autoimmune preventive activity.

To determine which splenic T cell subset, $CD4^+$ or $CD8^+$ population, mediates the prevention, the equivalent numbers of $CD4^+$ or $CD8^+$ splenic T cells were inoculated 2 days after TLI (Fig. 8). $CD4^+$ cells completely prevented the disease development, whereas $CD8^+$ cells did not.

Strain difference in the susceptibility to TLI-induced autoimmune disease

TLI elicited gastritis in the BALB/c ($H\text{-}2^d$), A ($H\text{-}2^a$), and SWR ($H\text{-}2^q$) strains of mice at high histologic incidences (70 to 90%) with high titers of antiparietal cell autoantibodies (Fig. 9). In contrast to these three strains, C57Bl/6 ($H\text{-}2^b$), DBA/2 ($H\text{-}2^d$), and C3H ($H\text{-}2^k$) were resistant to the autoimmune gastritis, although the C3H strain developed low titers of antiparietal cell autoantibodies. The histologic incidence of gastritis in (C57BL/6 × BALB/c)F_1 mice was intermediate between the incidences in C57BL/6 and BALB/c. Mild epididymitis/orchitis was observed in 30% of SWR mice and 10 to 20% of A and BALB/c mice. One A mouse developed thyroiditis with thyroglobulin autoantibodies (x320 by ELISA); 15 to 20% of A and BALB/c and 50% of SWR developed significant titers of antithyroglobulin autoantibodies (80 to 160 by ELISA). Significantly high titers (i.e., higher than the 95th percentile of the titers in normal nonirradiated mice) of IgG autoantibodies against ssDNA developed in ~50, ~60, and ~80% of BALB/c, A, and SWR strains, respectively, after TLI. Titers of IgG autoantibodies specific for dsDNA were not significantly high in these strains. Serum concentration of immune complexes was significantly high (i.e., higher than the 95th percentile of the concentrations in normal nonirradiated mice) in 40 to 50% of the A and SWR strains given TLI but with no histologic evidence of immune complex glomerulonephritis or vasculitis.

Discussion

Inflammatory lesions observed after TLI in the gastric mucosa, thyroid glands, and testes were T cell-mediated autoimmune diseases, because $CD4^+$ T cells could adoptively transfer the lesions to syngeneic athymic nude mice

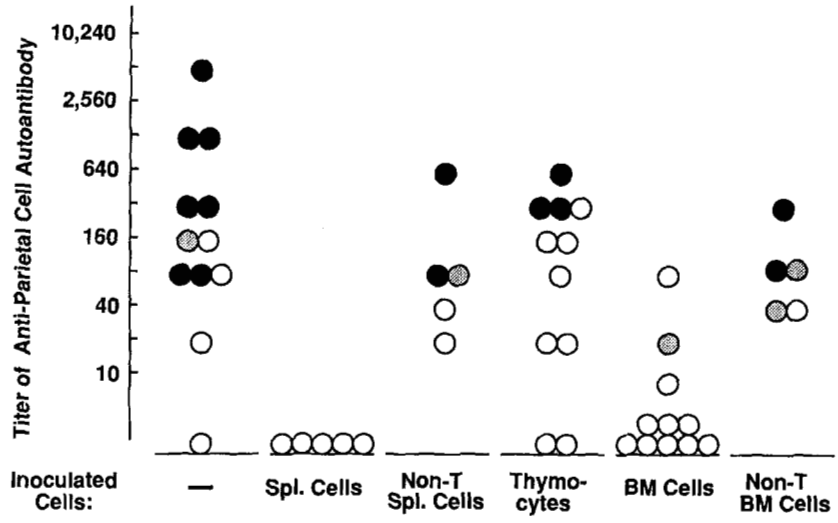

FIGURE 7. Prevention of autoimmune disease by inoculating spleen cell, thymocyte, or BM cell suspensions from syngeneic nonirradiated mice. Male BALB/c mice (3 mo old) were given TLI and inoculated 2 days later with normal BALB/c spleen cells (1×10^8), spleen cells treated with anti-Thy-1 plus complement ($\sim 6 \times 10^7$), thymocytes (1×10^8), BM cells (3×10^7), or BM cells treated with anti-Thy-1 plus complement ($2.5–3 \times 10^7$). The mice were histologically and serologically examined 4 mo later. ○, ●, ●; See Figure 1.

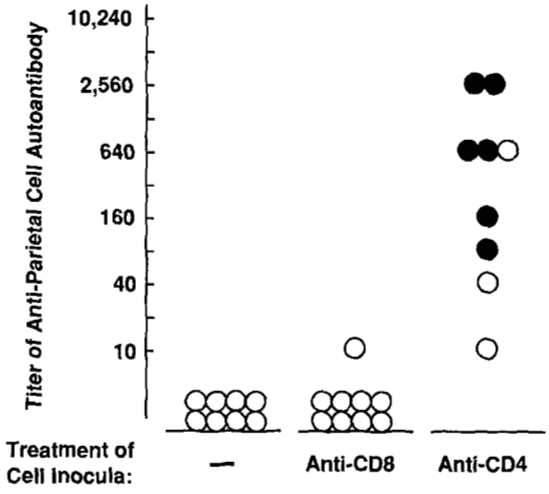

FIGURE 8. Prevention of autoimmune disease by inoculating splenic $CD4^+$ cells from syngeneic nonirradiated mice. Male BALB/c mice (3 mo old) given TLI were inoculated with normal BALB/c spleen cell suspensions treated with anti-CD4 or anti-CD8 Ab and complement then examined 4 mo later for histologic and/or serologic development of autoimmune gastritis. To inoculate an equivalent number of T cells, 1×10^8 or 5×10^7 spleen cells were treated with anti-L3T4 or anti-Lyt-2, respectively, because the number of $CD4^+$ cells in normal spleen is roughly twice the number of $CD8^+$ cells in BALB/c mice. As control, 5×10^7 cells were treated with complement only and i.v. inoculated. ○, ●; See Figure 1.

with resulting histologic damage of the corresponding organs and appearance of specific autoantibodies in the circulation. These autoimmune diseases were similar in immunopathology to human organ-specific autoimmune diseases, such as autoimmune gastritis with pernicious anemia, Hashimoto's autoimmune thyroiditis, or autoimmune orchitis in male infertility (1).

For the following reasons, TLI appears to affect primarily the T cell immune system not the target organs in eliciting autoimmune disease; i.e., 1) shielding the target organs (e.g., testes) during TLI did not prevent autoimmune disease, and irradiation of the target organ alone (e.g., stomach) failed to induce autoimmunity; 2) irradiation of both the thymus and the peripheral lymphoid tissues was required for efficient induction of autoimmune disease; 3) TLI depleted mature T cells from the thymus (40) and the periphery, and inoculation of normal mature thymocytes/T cells effectively prevented the autoimmune disease; and 4) similar organ-specific autoimmune diseases including gastritis, thyroiditis, and orchitis can be induced in normal mice by preparing a similar immunologic condition as in TLI without manipulating the target self-Ags, e.g., transplanting x-irradiated thymi into syngeneic athymic nude mice or mice depleted of T cells by Tx and x-irradiation at a T cell-depleting dose (12).

The finding that Tx and subsequent TLI could produce autoimmune disease indicates that TLI elicits pathogenic self-reactive T cells from the peripheral T cell pool. Penhale et al. (41, 42) reported a similar finding with rats: Tx in young adults and subsequent four times of fractionated whole body irradiation (2.5 Gy each every two weeks) induced chronic autoimmune thyroiditis and pancreatic insulitis/insulin-dependent diabetes mellitus in selected strains of rats. The irradiation on non-Tx rats elicited marginal pathologic changes in the thyroid (41). In our experiments, the incidence/severity of autoimmune disease in Tx-TLI mice was significantly lower than non-Tx mice given TLI (Fig. 4). In the non-Tx TLI mice, the T cells regenerating from the irradiated thymus may predominantly contain the pathogenic self-reactive T cells due to insufficient clonal deletion or defective production of mature $CD4^+$ cells with autoimmune preventive activity (see below) or both (12); the self-reactive T cells thus produced

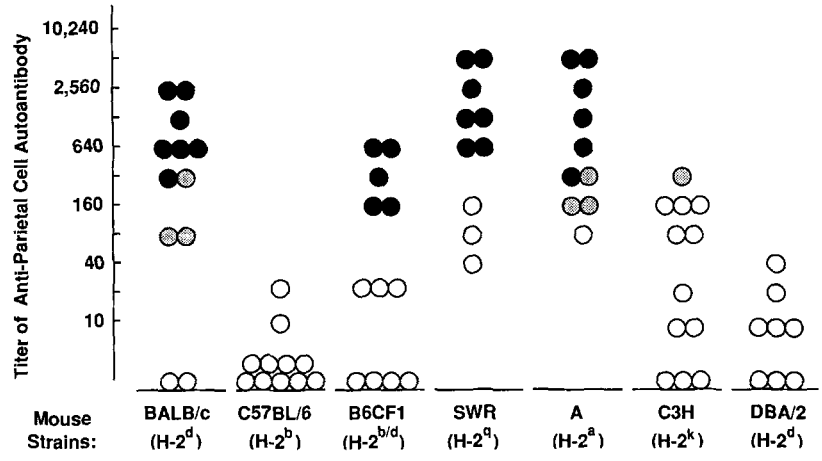

FIGURE 9. Strain difference in susceptibility to TLI-induced gastric autoimmunity. Male mice (3 mo old) of respective strains received TLI and were examined 6 mo later for histologic and/or serologic occurrence of gastric autoimmunity. BALB/c group is that shown in Figure 1. ○, ●, ●; See Figure 1.

may mediate the autoimmune responses synergistically with those derived from the peripheral T cell pool.

Inoculation of $CD4^+$ T cells prepared from syngeneic nonirradiated mice effectively prevented TLI-induced autoimmunity, whereas $CD8^+$ cells did not. The autoimmune inhibitory $CD4^+$ cells appear to control $CD4^+$ pathogenic self-reactive T cells at an early stage of their proliferation/differentiation, because the T cell inoculation immediately after TLI was effective in disease prevention but the inoculation 1 mo or later (when the autoimmunity became histologically and serologically evident [Fig. 1]) was not (Fig. 6). Similar $CD4^+$ cell-dependent autoimmune prevention, effective in early stages of disease progression, has been documented in induced and spontaneous models of various organ-specific autoimmune diseases (43–49). Furthermore, organ-specific autoimmune diseases including gastritis, thyroiditis, and orchitis can be induced in normal rodents (e.g., BALB/c mice) by eliminating a $CD4^+$ subpopulation defined by the expression of particular cell surface molecules (e.g., CD5) (11, 47–49).

Taken together, these findings suggest that the mature $CD4^+$ thymocytes/T cells with the autoimmune inhibitory activity may be more sensitive to TLI than the self-reactive $CD4^+$ T cells; TLI may eliminate/reduce the number of the autoimmune inhibitory T cells in the periphery or their generation in the thymus, thereby tipping the balance toward an expansion of the self-reactive T cells and causing autoimmune disease. The two $CD4^+$ populations might be different in the expression of cell surface receptors for certain lymphokines and/or in the pattern of lymphokine secretion (49–51); and lymphokine(s) might mediate their interaction in maintaining self-tolerance. This issue is currently under investigation.

It is paradoxical that TLI can induce allograft tolerance on the one hand but break self-tolerance on the other. This paradox is similar to the one with CsA, which not only establishes nearly permanent immunologic tolerance to allografts but also causes similar organ-specific autoimmune diseases in mice (e.g., autoimmune gastritis in BALB/c mice) or syngeneic GvHD in x-irradiated and BM-transplanted mice or rats (27, 52–55). Inoculation of normal syngeneic $CD4^+$ T cells prevented these autoimmunities (27, 54). The syngeneic GvH-like disease in the *nu/nu* mice transferred with T cell suspensions from TLI mice (see *Results*) might also have a common pathogenetic basis as the CsA-induced syngeneic GvH-like disease in which transfer of T cell suspensions from CsA-treated mice elicited wasting disease in syngeneic irradiated mice even though the donors were not afflicted with GvHD (53, 54). Similar alteration in the thymic architecture (e.g., loss of the thymic medulla) and the composition of thymocyte subsets (e.g., reduction of mature thymocytes) after TLI or CsA treatment may contribute to the development of common autoimmunity by similarly affecting thymic or peripheral control of autoimmune T cells (27, 31, 40, 55–57).

The autoimmune responses elicited by TLI affected a particular spectrum of organs. Furthermore, the susceptibility to individual autoimmune diseases was different among the mouse strains given TLI. The difference between susceptible BALB/c and resistant DBA/2 (which share the *d* haplotype of MHC [58]) or between susceptible A and resistant C3H (which share the *k* haplotype of class II MHC [58]) indicates that the host class II MHC gene by itself is unable or insufficient, at least in these strains, to determine the susceptibility. This difference is not due to genetic difference in the radiosensitivity of the target organs, because the organ susceptibility in TLI-induced autoimmune disease was similar to the one observed with autoimmune induction by other procedures for manipulating the thymus/T cells not the target organs (11, 12, 27, 59, 60).

These findings taken together indicate that when the radiation-induced T cell abnormality elicits autoimmune responses, the host genetic factors significantly contribute to determining the specificity/intensity of the autoimmune responses. Indeed, in contrast to the frequent gastric autoimmunity in mice after TLI, TLI predominantly elicited thyroid autoimmunity in normal strains of rats (e.g., the

PVG strain) even with shielding of the thyroid gland from irradiation (N. Sakaguchi and S. Sakaguchi, unpublished data). Further immunologic and genetic study of this radiation-induced autoimmune disease in humans (61, 62) and animals would contribute to our understanding of the roles of environmental, immunologic, and genetic factors in autoimmune pathogenesis.

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