Thymus and Autoimmunity: Capacity of the Normal Thymus to Produce Pathogenic Self-Reactive T Cells and Conditions Required for their Induction of Autoimmune Disease

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Summary

BALB/c athymic *nu/nu* mice spontaneously developed organ-specific (gastritis, thyroiditis, oophoritis, or orchitis) and systemic (arteritis, glomerulonephritis, and polyarthritis) autoimmune diseases when transplanted with neonatal BALB/c thymuses. Transplantation of thymuses from adult BALB/c mice was far less effective in inducing histologically evident organ-specific autoimmune disease in *nu/nu* mice. Autoimmune disease developed, however, when adult thymuses were irradiated at a T cell–depleting dose before transplantation. Engrafting newborn thymuses into BALB/c mice T cell depleted by thymectomy, irradiation, and bone marrow transplantation produced similar organ-specific autoimmune disease as well, but thymus engrafting into T cell–nondepleted BALB/c mice (i.e., mice thymectomized as adults, but not irradiated) did not, despite the fact that transplanted thymuses grew well in both groups of mice.

The mice with organ-specific autoimmune disease produced autoantibodies specific for the respective organ components, such as gastric parietal cells, thyroglobulins, oocytes, or sperm. The thymus-transplanted *nu/nu* mice also had hypergammaglobulinemia and developed anti-DNA autoantibodies, rheumatoid factors, and immune complexes in the circulation.

These results indicate that: (*a*) the thymus of a murine strain that does not develop spontaneous autoimmune disease can produce pathogenic self-reactive T cells that mediate organ-specific and/or systemic autoimmune diseases; and (*b*) such self-reactive T cells, especially those mediating organ-specific autoimmune disease, spontaneously expand and cause autoimmune disease when released to the T cell–deficient or –eliminated periphery.

Autoimmune diseases are divided into organ-specific (e.g., Hashimoto's thyroiditis and autoimmune gastritis with pernicious anemia) or non–organ-specific (systemic; e.g., SLE and rheumatoid arthritis), depending on whether autoimmune responses are directed to an antigen(s) confined to a particular organ, or widely distributed in the body (1, 2). In humans as well as animal models, one organ-specific autoimmune disease is frequently associated with another, e.g., pancreatic insulitis in insulin-dependent diabetes, thyroiditis, and gastritis (3–7); likewise, a number of clinical features are shared by SLE and rheumatoid arthritis (1, 2, 8). This suggests a common pathogenetic basis for each spectrum of autoimmune diseases.

T cells play a pivotal role in generating various autoimmune diseases in humans and animals (9–14). The existence of potentially self-reactive T cells in the normal immune system has been suggested for organ-specific autoimmune diseases (15–17), but remains controversial for systemic ones (18). Recent studies have demonstrated that T cells reactive with self-antigens expressed in the thymus can be clonally deleted (19, 20), but how the thymus deals with T cells specific for self-antigens expressed outside the thymus remains to be determined.

A critical issue in elucidating the pathogenetic mechanism of autoimmune disease is to determine whether the thymus of a normal individual can produce T cells mediating organ-specific and/or systemic autoimmune disease, and, if so, what conditions are required for their expansion and induction of autoimmune disease. In this report, we show that, when congenitally T cell–deficient athymic nude (*nu/nu*) mice or euthymic mice T cell depleted by thymectomy and irradiation are engrafted with syngeneic thymuses, they spontaneously develop various organ-specific and systemic autoimmune diseases.

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Materials and Methods

Mice

BALB/c *nu/nu* and *nu/+* mice (6–8 wk old) were purchased from Life Sciences, St. Petersburg, FL. Euthymic fetuses or newborns were obtained by breeding female *nu/+* mice with male *nu/+* mice. To prepare T cell–depleted *nu/+* mice, female *nu/+* mice were thymectomized at 6 wk of age, irradiated 2 wk later at 900 rad from a ^{137}Cs source (81.3 rad/min; Gammacell 40 irradiator; Atomic Energy of Canada, Ottawa, Canada), and inoculated with 5×10^6 syngeneic bone marrow cells treated with anti-Thy-1.2 plus rabbit complement.

Thymus Transplantation

Thymuses were engrafted under the renal capsule as previously described (21). Thymuses irradiated before engrafting either received 900 rad from a ^{137}Cs source after removal from newborn or 7-d-old *nu/+* hosts, or were removed 2 d after 900-rad whole body irradiation of adult *nu/+* mice.

ELISA

Antibodies against Double- or Single-stranded DNA (ds- or ssDNA),[1] TNP haptens, or IgG (RF). 5 μg/ml ds- or ssDNA (22), 10 μg/ml TNP-BSA (23), or 5 μg/ml affinity-purified mouse IgG (Sigma Chemical Co., St. Louis, MO) (8) in PBS, pH 7.2, was used for overnight coating of ELISA plates (Flow Laboratories, McLean, VA). Test sera were diluted to 1:20 for RF assay, 1:40 for anti-DNA assay, or 1:80 for anti-TNP assay. Antigen-coated plates were blocked for 1 h with PBS containing 0.05% Tween 20, 0.02% NaN_3, and 0.1% BSA, incubated for 1 h at room temperature with appropriately diluted test sera, washed with PBS-0.05% Tween 20, 0.02% NaN_3, and incubated for 1 h with 1 μg/ml alkaline phosphatase (ALP)-conjugated anti-mouse IgG or IgM (for RF assay) (Southern Biological Technology, Birmingham, AL). Absorbance at 405 nm was measured by a MR580 ELISA reader (Dynatech, Alexandria, VA) after 30-min color development with 1 mg/ml *p* -nitrophenyl disodium hexahydrate (Sigma Chemical Co.) in 10% diethanolamine buffer, pH 9.8. RF and anti-DNA titers were expressed as units when the absorbance of 1:20 or 1:40, respectively, diluted standard pooled serum from ~4-mo-old MRL/MpJ-*lpr/lpr* mice (8), provided by Dr. E. Alexander of the Johns Hopkins University (Baltimore, MD), was arbitrarily assumed to be 100 U. In anti-TNP assay, the absorbance of a 1:80-diluted BALB/c serum prepared by immunizing with TNP-KLH was assumed to be 100 U.

Anti–Gastric Parietal Cell Autoantibody. Details of this method were previously described (7).

Serum Concentration of Ig. Plates coated with 1 μg/ml goat anti–mouse IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) were incubated with 1:1,000 or 1:5,000 diluted test sera, then with ALP-conjugated goat anti-IgG or anti-IgM (Southern Biological Technology), and color developed as described above. IgG and IgM concentrations of test sera were determined from a standard curve made by incubating with known concentrations of affinity-purified IgG and IgM (Sigma Chemical Co.).

Immune Complexes (IC). The solid-phase anti-C3 assay of Pereira et al. (24) was modified for ELISA, and serum IC concentrations were expressed as micrograms aggregated gammaglobulin equivalent per milliliter by referring to the standard curve of the binding of aggregated gammaglobulins prepared by heating mouse IgG (Sigma Chemical Co.).

Criteria for Grading Autoimmune Disease

Gastritis and oophoritis were assessed by macroscopic and histological examination, as previously described (17). Histological severity of glomerulonephritis was graded on a 0 to 2+ scale based on the intensity and extent of histopathologic changes (8, 25): 0 = kidneys without glomerular lesions; 1+ = mild lesions (increased mesangial matrix, mesangial/glomerular cellularity, crescent formation, or presence of inflammatory exudates and capsular adhesions; no noticeable tubular casts); 2+ = severe lesions (glomerular architecture obliterated in >70% of glomeruli with extensive tubular cast formation). Vasculitis was graded 0 to 2 based on histological types and severity of arterial lesions in the kidneys (25, 26; see Results for details). Arthritis was histologically assessed when joint swelling of fore and/or hind legs was macroscopically evident: 1+ synovitis with pannus formation but intact cartilages/bones; 2+ = arthritis with erosion of cartilages/bones or fibrous ankylosis in any joints.

Immunofluorescence (IF) Test

To detect ICs deposited in renal glomeruli or vascular walls, cryostat sections of kidneys were stained with FITC-labeled goat anti-IgG (Tago Corp., Burlingame, CA), anti-IgM (Tago Corp.), or anti-C3 (Cappel Laboratories, West Chester, PA). Indirect IF test for detecting tissue- or organ-specific autoantibodies was performed as previously described (7, 17).

Other Methods

Organs and tissues were processed for staining with hematoxylin and eosin (H&E) or periodic acid Shiff (PAS). Proteinuria was assessed by Uristix (Miles Laboratories Inc., Elkhart, IN).

Results

BALB/c nu/nu Mice Spontaneously Developed Autoimmune Disease when Engrafted with Newborn nu/+ Thymuses. BALB/c *nu/nu* mice were engrafted with newborn (0 or 1 d old) *nu/+* thymuses at 6–8 wk of age, observed for 5 mo, and examined for histological and/or serological occurrence of autoimmune disease (Table 1). 43 mice (96%) survived >4 mo; four mice were killed at 4.5–5 mo because of anasarca and splenomegaly (two mice) or prolapsus ani (two mice). Organ-specific autoimmune disease(s) was histologically observed in 86 and 58% of the thymus-grafted female and male *nu/nu* mice, respectively; more than one organ was affected in 17% of the former. 31% of the thymus-grafted *nu/nu* mice developed histologically demonstrable vasculitis, glomerulonephritis, or arthritis, or combinations thereof; 30% of mice with this systemic autoimmune disease also showed organ-specific autoimmune disease. No histologically evident autoimmune disease was observed in age-matched untreated *nu/+* or *nu/nu* mice (80% of the latter survived to 6 mo of age; 20% were killed early because of emaciation).

Histological Analysis. The afflicted organs in organ-specific autoimmune disease were massively infiltrated by mononuclear cells; the target cells (gastric parietal cells, thyroid epithelial

[1] *Abbreviations used in this paper:* ALP, alkaline phosphatase; BMT, bone marrow transplantation; dsDNA, double-stranded DNA; H&E, hematoxylin and eosin; IC, immune complexes; IF, immunofluorescence; Ir, irradiation; PAS, periodic acid Schiff; SSDNA, single-stranded DNA; Tx, thymectomy.

Table 1. *Induction of Autoimmune Disease in BALB/c nu/nu Mice by Engrafting Newborn Thymuses*

Experimental group	Thymus-grafted hosts*	Incidence of autoimmune disease‡					
		Gastritis	Oophoritis or orchitis	Thyroiditis	Vasculitis	Glomerulonephritis	Arthritis
A	BALB/c *nu/nu* (F)	22/35 (32/35)	9/35 (8/35)	2/35 (3/35)	8/35 (1°; 5 2°; 3)	4/35 (1°; 2 2°; 2)	2/35 (2°; 2)
B	BALB/c *nu/nu* (M)	7/12 (9/12)	1/12 (2/12)	0/12 (1/12)	3/12 (1°; 2 2°; 1)	1/12 (2°; 1)	0/12

* Female (F) or male (M) BALB/c *nu/nu* mice (6–8 wk old) were engrafted with newborn BALB/c thymuses. The mice were killed 4.5–5 mo later for histological examination and check of autoantibodies.
‡ Incidence of histologically evident autoimmune disease is shown (see Figs. 1–3 and reference 17). Incidence of autoantibody-positive mice, assessed by IF test at 1:10 dilution of test sera, is shown in parentheses for gastritis, thyroiditis, oophoritis, or orchitis (17). The number of mice with grade 1 (1°) or grade 2 (2°) of vasculitis, glomerulonephritis, or arthritis is shown in parentheses.

cells, or oocytes) were specifically destroyed (see reference 17 for macroscopic view, histology, and autoantibodies demonstrated by indirect IF test).

Two types of vascular involvement were noticed in small- and medium-sized arteries, most noticeable in the kidney and then salivary gland (Fig. 1). 14% of *nu/nu* mice with thymus grafts showed perivascular accumulation of mononuclear cells, little cellular infiltration into arterial walls with slight disruption of the walls, and no IC deposition (Fig. 1, *A* and *B*). 9% showed not only perivascular accumulation of mononuclear cells and neutrophils, but also cellular infiltration into arterial walls, leukocytoclasis, and fibrinoid necrosis (Fig. 1 *C*). Although we designated the former grade 1 and the latter grade 2 by emphasizing arterial wall damage (Table 1), it remains to be determined whether these two types represent transitional stages from one to the other (26).

Two mice showed severe proteinuria (~2,000 mg/ml) and histologically severe (grade 2) glomerulonephritis of a chronic obliterative form with accumulation of amorphous PAS-positive materials in the mesangial matrix (Fig. 2, *A* and *B*), granular deposition of IgG and C3 in the glomeruli (Fig. 2, *C* and *D*), and extensive protein casts in renal tubules. These two mice also showed grade 2 arteritis in the kidney (Fig. 1 *C*). Deposition of IgM was observed by IF test in the glomeruli of untreated *nu/nu* mice, as reported by others (27, 28), but their glomeruli were histologically normal.

Arthritis symmetrically involved small and large joints of both fore and hind feet (Fig. 3 *A*). Synovitis with pannus formation observed in early lesions (Fig. 3 *B*) appeared to proceed to cartilage and bone erosion, resulting in fibrous ankylosis of the joints (Fig. 3 *C*). Subcutaneous nodules, vasculitis of small vessels, and inflammation of the surrounding muscle and tendon were also observed.

Serological Analysis. Serum titers of various autoantibodies, IgG concentration, or IC levels in the thymus-transplanted female *nu/nu* mice in Table 1 were assessed and compared with control untreated female *nu/nu* or *nu/+* mice, or ~4-mo-old MRL/Mp-*lpr/lpr* mice that spontaneously develop various SLE-like autoantibodies as well as IC-mediated glomerulonephritis, arteritis, and arthritis (8; and see Discussion) (Fig. 4). IgG concentration, IC levels, titers of organ-specific autoantibodies (especially those specific for gastric parietal cells), and autoantibodies against dsDNA, anti-TNP antibodies, or RF, were significantly high in the *nu/nu* mice with thymus grafts. One mouse with grade 2 arteritis had the highest titer of RF and anti-dsDNA autoantibodies, and three mice with arteritis had high levels of serum ICs. Untreated *nu/nu* mice developed autoantibodies against ssDNA with aging (data not shown), but no significant titer of anti-dsDNA autoantibodies.

Engrafting of Adult nu/+ Thymuses Was Less Efficient in Inducing Organ-specific Autoimmune Disease in nu/nu Mice. To examine whether the successful induction of autoimmune disease is unique to newborn thymus transplants, BALB/c *nu/nu* mice were engrafted with thymuses from *nu/+* mice at various ages: two thymuses from *nu/+* or *+/+* fetuses on day 14 of gestation (day 6); one (two lobes) from 0-d-old (newborn within 24 h of birth) or 7 d-old *nu/+* mice; or a half thymus (one lobe), cut into three to four pieces, from 8-wk-old *nu/+* mice. They were examined 3 mo later for histological and serological development of autoimmunity (Table 2). 50 and 70% of the mice transplanted with fetal or newborn thymuses, respectively, developed histologically demonstrable gastritis and/or oophoritis with respective autoantibodies. Thymus engrafting from 7-d-old or adult *nu/+* mice was far less effective in inducing these autoimmune diseases, although 40–50% showed high titers (≥640 by ELISA) of anti–parietal cell autoantibodies of IgG isotype. Serum IgG levels and titers of anti-dsDNA or anti-TNP antibodies were equally high (comparable with the thymus-grafted *nu/nu* mice

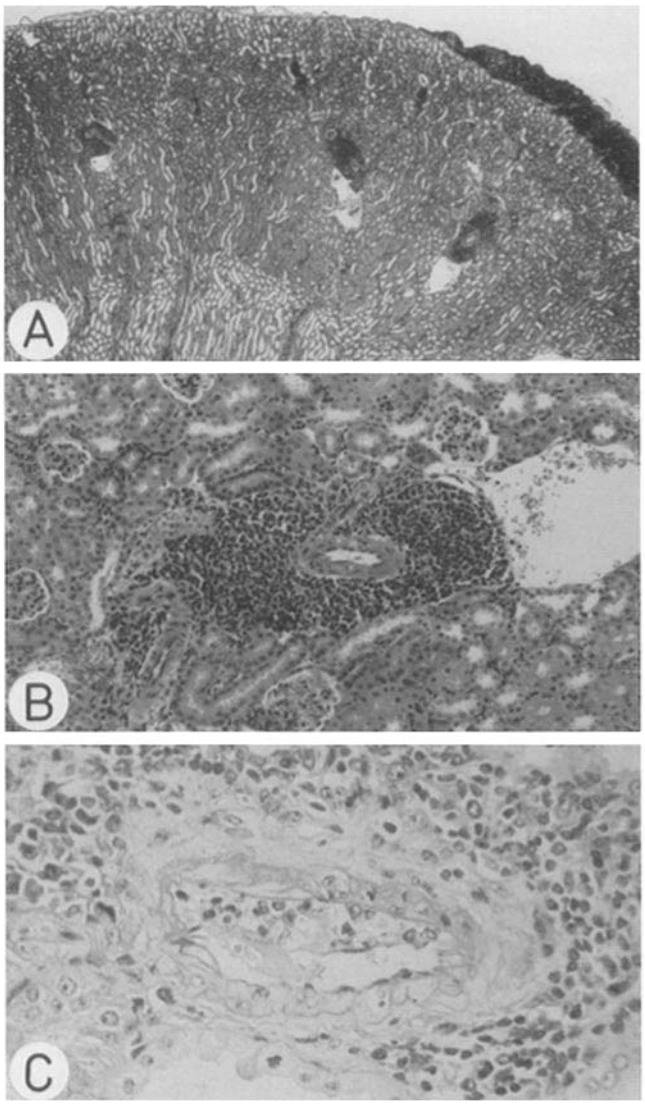

Figure 1. Vasculitis. (*A*) Vascular lesion in the kidney of thymus-transplanted *nu/nu* mice. Small- and medium-sized muscular arteries are affected. Transplanted thymus is also shown (H&E staining; ×40). (*B*) Higher magnification of the lesion shown in *A*. Perivascular accumulation of mononuclear cells is prominent, but disruption of arterial wall is slight (grade 1) (H&E staining; ×180). (*C*) Arteritis with damage of the media and intima (grade 2) (PAS staining; ×200). Subendothelial deposition of PAS-positive material is seen.

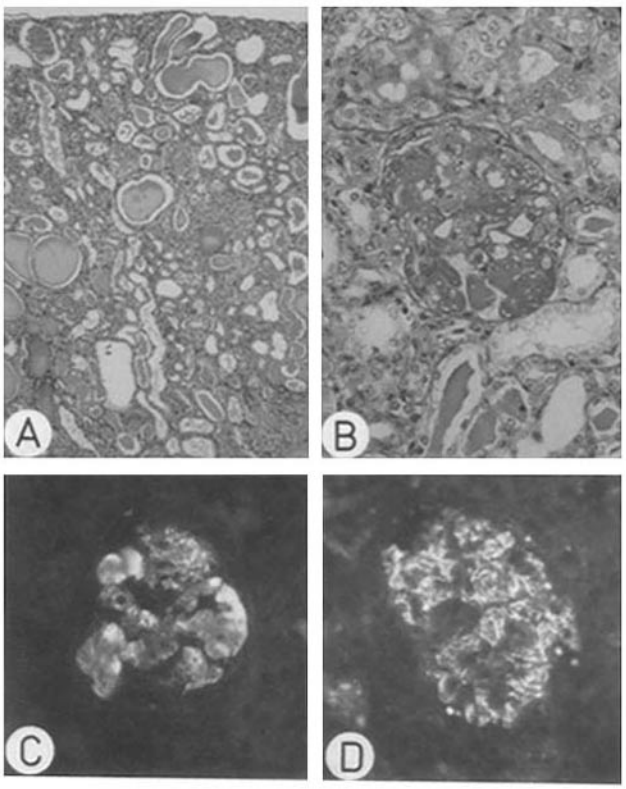

Figure 2. Glomerulonephritis. (*A*) Glomeruli are extensively damaged and protein casts are seen in the tubules (grade 2) (H&E staining; ×40). Note cellular infiltration into the interstitial tissue. (*B*) Affected glomerulus (PAS staining; ×250). (*C*) IF staining of IgG deposition (×250). (*D*) IF staining of C3 deposition (×250).

in Fig. 4) in *nu/nu* mice with thymuses of any age; histological incidence of systemic autoimmune disease appeared to be little influenced by the age of thymus donors.

Transplantation of Irradiated Thymuses from nu/+ Mice at Any Age Induced Autoimmune Disease in nu/nu Mice. To examine effects of T cell depletion from thymus grafts before transplantation, thymuses were irradiated at 900 rad and then engrafted into *nu/nu* mice (Table 2). In contrast to nonirradiated thymuses (see above), engraftment of irradiated thymuses from 0-d, 7-d, or 2-mo-old *nu/+* mice equally produced histologically evident organ-specific autoimmune disease(s) in ~80% of the *nu/nu* mice. Two mice developed mild insulitis (see reference 7 for histology). Serum IgG levels and autoantibody titers were not significantly different between the *nu/nu* mice with irradiated or nonirradiated thymus grafts.

Development of Organ-specific Autoimmune Disease Required Peripheral T Cell Depletion before Engrafting nu/+ Thymuses. To determine whether spontaneous development of autoimmune disease after thymus grafting is unique to *nu/nu* mice, BALB/c newborn thymuses were engrafted into T cell–depleted BALB/c mice (prepared by thymectomy [Tx] as adults, irradiation [Ir], and bone marrow transplantation [BMT]), BALB/c thymectomized without T cell depletion, and normal BALB/c (Table 3). At 3 mo, the thymus grafts grew well under the renal capsule of these mice, except normal BALB/c. Organ-specific autoimmune diseases were histologically found in 75 and 50% of the *nu/nu* or Tx-Ir-BMT-BALB/c, respectively, with high titers of anti–parietal cell autoantibodies (640–40,960 by ELISA), but not in the Tx- or normal BALB/c, in which no anti–parietal cell titer was detected (<10 by ELISA). Serum IgG levels and anti-dsDNA titers were high (15–30 mg/ml and 10–35 U, respectively) in the thymus-grafted *nu/nu*, Tx-Ir-BMT-, or Tx-BALB/c mice compared with normal BALB/c with thymus grafts (3–8 mg/ml and <5 U, respectively).

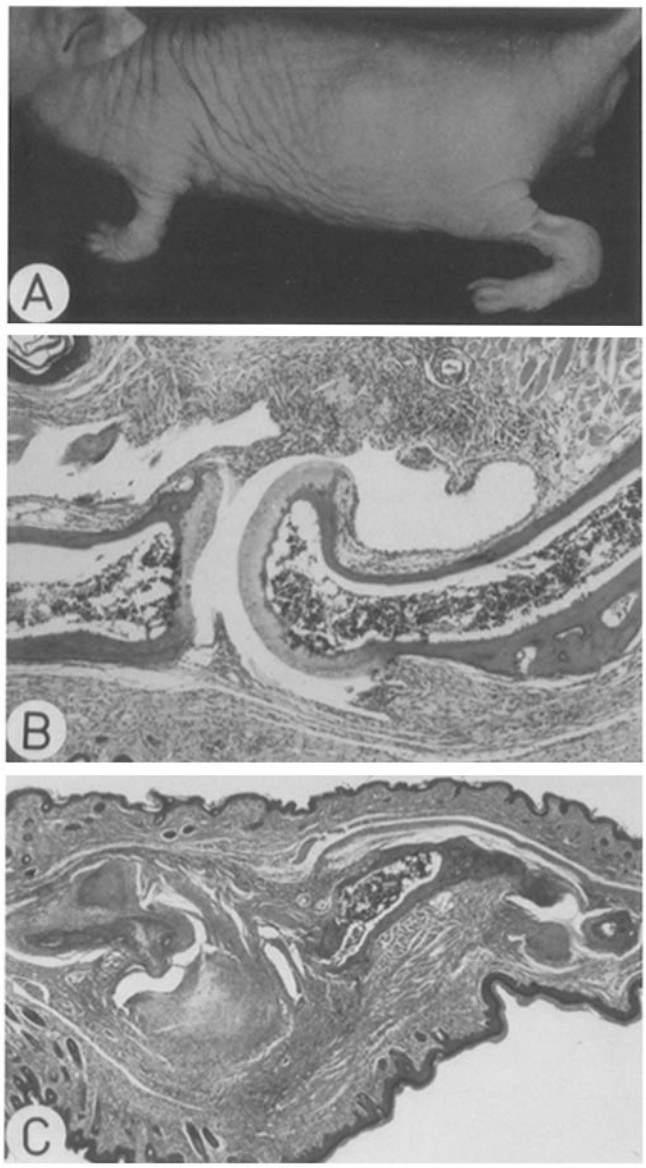

Figure 3. Arthritis. (*A*) Joint swelling in fore and hind legs. (*B*) Early lesions in an interphalangeal joint (grade 1) (H&E staining; ×80). Note synovial inflammation and pannus formation. (*C*) Late lesions with destruction of cartilage and absorption of bone (grade 2) (×40).

Discussion

When T cell–deficient or –depleted BALB/c mice were engrafted with syngeneic thymuses, they spontaneously developed various organ-specific (gastritis, thyroiditis, insulitis, oophoritis, or orchitis) and systemic (arteritis, glomerulonephritis, and arthritis) autoimmune diseases that were immunopathologically similar to those in humans (1).

In the organ-specific autoimmune diseases shown here, T cells produced/released by the thymus grafts appeared to exert antigen-specific help on the host-derived autoantibody-forming B cells and/or conduct cell-mediated immune reactions towards specific self-antigens, since these autoimmune diseases could be adoptively transferred by T cells alone to naive *nu/nu* mice in a disease-specific manner (17; and manuscript in preparation). There was a difference in the incidence of organ-specific autoimmune diseases dependent upon the age of the thymus donors; this age-dependent difference was abolished by irradiation (Table 2). Fetal/newborn thymuses and regenerating thymuses after irradiation contained few "mature" thymocytes (i.e., few $CD4^+$ or $CD8^+$ single-positive thymocytes; 29, 30). "Mature" thymocytes were also depleted by administration of cyclosporin A, and transplantation of thymuses from cyclosporin A–treated adult mice produced similar organ-specific autoimmune disease in syngeneic *nu/nu* mice (7, 21). Inoculation of thymocyte suspensions from normal adult mice could inhibit the development of organ-specific autoimmune disease, but those from newborn or cyclosporin A–treated mice could not (21, 31). Furthermore, depletion of T cells from the periphery by irradiation appeared to prompt peripheral expansion of self-reactive T cells upon release from the thymus grafts (Table 3). These findings, when taken together, suggest that thymuses of any age can produce pathogenic self-reactive T cells eliciting organ-specific autoimmune disease; however, "mature" thymocytes/T cells, or certain T cells in the "mature" subset, may inhibit peripheral proliferation/activation of the self-reactive T cells.

In the development of SLE-like autoimmune disease, it is unlikely that organ-specific self-antigens, such as parietal cell or oocyte antigens, formed the main pathogenic ICs, since BALB/c *nu/nu* mice inoculated with a *nu/+* splenic T cell subset developed the same spectrum of organ-specific autoimmune diseases with comparative titers of organ-specific autoantibodies, but did not show histologically evident vasculitis or glomerulonephritis, nor a significant level of circulating ICs (17). In the thymus-grafted *nu/nu* mice, T cell–mediated polyclonal activation of host B cells, illustrated by hypergammaglobulinemia and spontaneous appearance of anti-TNP antibodies, presumably played a key role in the development of SLE-like autoantibodies (such as against dsDNA molecules) and resulting pathogenic ICs (32–35). The role of T cell–mediated polyclonal B cell activation has been implicated in systemic autoimmunity of mice with chronic graft-vs.-host disease and MRL/Mp-*lpr/lpr* mice; both develop arteritis, IC-mediated glomerulonephritis, and polyarthritis, immunopathologically similar to those shown here (Fig. 4) (8, 25, 26, 36–38). In these models, T cells reactive with allogenic class II MHC antigens (39), or perhaps abnormally reactive with self-class II MHC antigens (40–42), appear to stimulate B cells polyclonally through direct contact and/or via various B cell stimulatory lymphokines (39, 43, 44). In our experiment, inoculation of whole spleen cells from normal adult BALB/c *nu/+* mice did not cause graft-vs.-host disease or systemic autoimmunity in BALB/c *nu/nu* mice (17), indicating no significant histoincompatibility between *nu/nu* and congenic *nu/+* mice. Grafted thymuses regenerating from presumed structural damage upon heterotopic transplantation (45) might produce, or fail to delete, T cells with nonphysiological reactivity to self-class II MHC or related self antigens (19, 46, 47). This issue is currently under investigation.

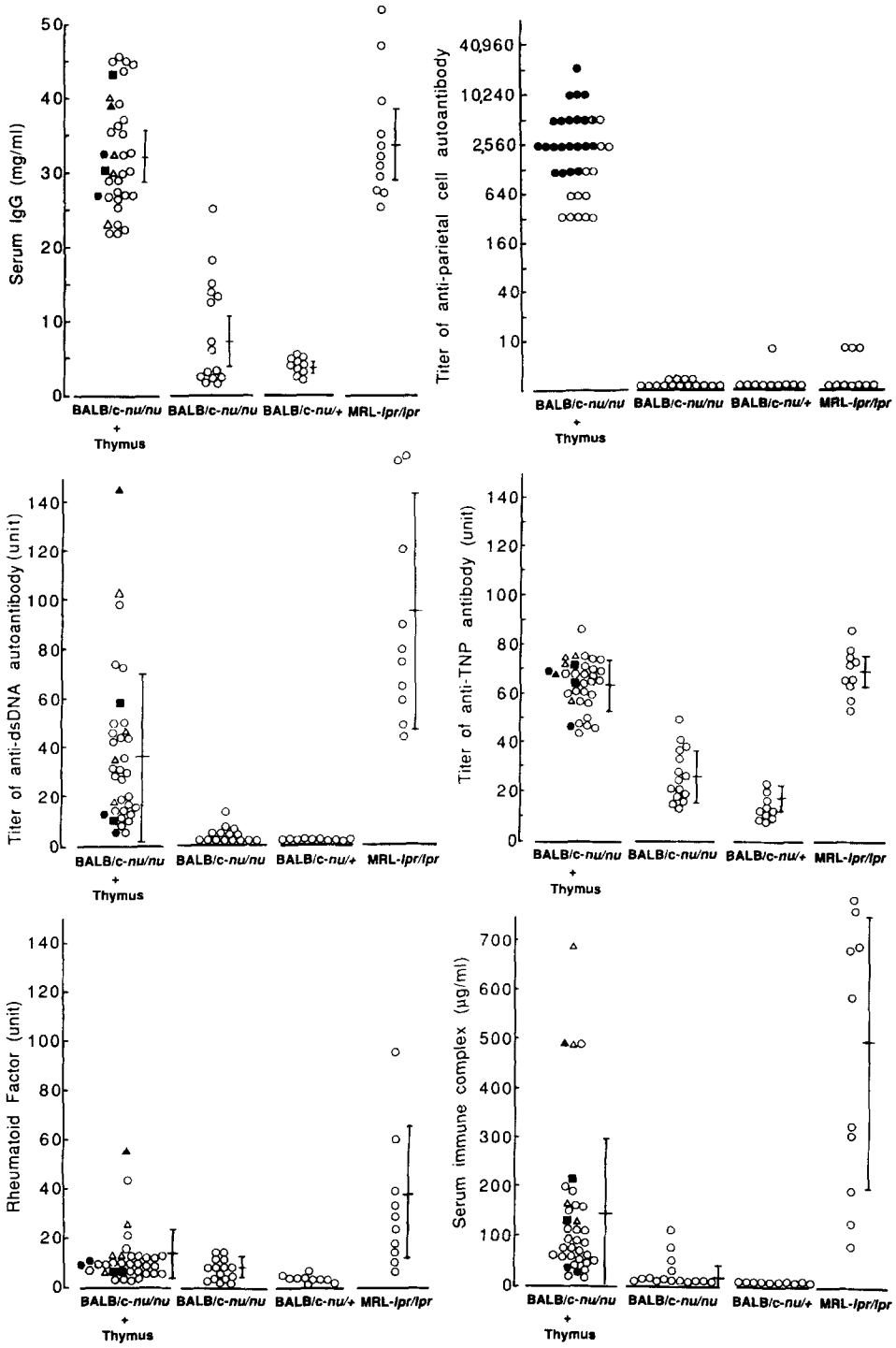

Figure 4. Serum IgG level, titers of anti-parietal cell autoantibody, anti-dsDNA autoantibody, anti-TNP antibody, RFs, and level of serum immune complexes are compared among BALB/c female *nu/nu* mice with thymus grafts (Table 1), age- and sex-matched BALB/c *nu/nu* or *nu/+* mice, and ~4-mo-old MRL-*lpr/lpr* mice. (●) Macroscopically evident gastritis; (⬢) 2° glomerulonephritis; (⬡) 1° glomerulonephritis; (▲) 2° arteritis; (△) 1° arteritis; (■) 2° arthritis. Two mice shown as having 2° glomerulonephritis also bore 2° arteritis. Bars indicate mean ± SD.

Thus, the thymus of BALB/c mice can produce T cells that mediate organ-specific and systemic autoimmune disease; however, the T cell self-reactivities responsible for organ-specific and systemic autoimmunity may differ or be subjected to a distinct thymic and/or peripheral control. The difference was also suggested by the finding that hypergammaglobulinemia and significantly high titers of anti-dsDNA antibodies developed, but no organ-specific autoantibodies were detected, in thymus-grafted Tx-BALB/c mice (Table 3, group C; and Results); furthermore, similar organ-specific autoimmune disease can be induced in BALB/c mice without systemic autoimmunity (7, 17). Low incidence of overt systemic autoimmune disease in the present experiments requires further study to clarify this issue.

Table 2. *Induction of Autoimmune Disease in BALB/c nu/nu Mice by Engrafting Irradiated or Nonirradiated Thymuses from BALB/c nu/+ Mice at Various Ages*

Experimental group	Age of donor mice	Treatment of thymus grafts*	Incidence of autoimmune disease‡						
			Gastritis	Oophoritis	Thyroiditis	Insulitis	Vasculitis	Glomerulonephritis	Arthritis
A	Day (−6)	-	3/8 (5/8)	1/8 (2/8)	0/8 (0/8)	0/8	1/8	0/8	0/8
B	Day 0	-	6/10 (8/10)	3/10 (4/10)	0/10 (0/10)	0/10	2/10	0/10	1/10
C	Day 7	-	1/10 (5/10)	0/10 (1/10)	0/10 (0/10)	0/10	1/10	0/10	0/10
D	2 mo	-	0/10 (4/10)	0/10 (0/10)	0/10 (0/10)	0/10	1/10	1/10	0/10
E	Day 0	irradiated	4/6 (6/6)	4/6 (4/6)	0/6 (0/6)	0/6	0/6	0/6	0/6
F	Day 7	irradiated	5/6 (6/6)	3/6 (4/6)	1/6 (1/6)	1/6	0/6	0/6	0/6
G	2 mo	irradiated	3/5 (5/5)	1/5 (2/5)	0/5 (0/5)	1/5	1/5	1/5	0/5

* Female BALB/c *nu/nu* mice (6–8 wk old) were engrafted with irradiated or nonirradiated thymuses from *nu/+* mice at indicated ages. The recipient *nu/nu* mice were killed 3 mo later for histological and serological examination.
‡ See legend for Table 1. A mouse in group G developed both 2° arteritis and 2° glomerulonephritis; vasculitis, glomerulonephritis, and arthritis in other groups were 1° in histological severity.

Table 3. *Induction of Autoimmune Disease in T Cell–deficient Mice by Engrafting Newborn Thymuses*

Experimental group	Thymus-grafted hosts*	Incidence of autoimmune disease‡				
		Gastritis	Oophoritis	Thyroiditis	Vasculitis	Arthritis
A	BALB/c *nu/nu*	7/12 (11/12)	4/12 (5/12)	1/12 (2/12)	2/12	1/12
B	BALB/c (Tx-Ir-BMT)	6/12 (8/12)	–§	0/12 (0/12)	0/12	0/12
C	BALB/c Tx	0/10 (0/10)	0/10 (0/10)	0/10 (0/10)	0/10	0/10
D	BALB/c	0/10 (0/10)	0/10 (0/10)	0/10 (0/10)	0/10	0/10

* Female BALB/c *nu/nu* mice (6–8 wk old) (group A), BALB/c mice (8 wk old) T cell depleted by Tx, Ir, and BMT (group B), thymectomized BALB/c mice (group C), or normal BALB/c (group D) were engrafted with newborn BALB/c thymuses. These mice were killed 3 mo later for histological and serological examination.
‡ See legend for Table 1. Two cases of vasculitis were 1° of histological severity; a case of arthritis was 2°.
§ Ovaries were destroyed by irradiation.

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