THYMUS DICTATES MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) SPECIFICITY AND IMMUNE RESPONSE GENE PHENOTYPE OF CLASS II MHC-RESTRICTED T CELLS BUT NOT OF CLASS I MHC-RESTRICTED T CELLS

By W. MARTIN KAST, LEO P. DE WAAL AND CORNELIS J. M. MELIEF

From the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Amsterdam, The Netherlands.

Cytotoxic T lymphocytes (CTL)[1] specific for viruses, haptens, and minor histocompatibility antigens are restricted to molecules encoded by class I MHC genes (1–4). CTL responses are regulated by class I gene products (H-2K, H-2D, and H-2L molecules in the mouse), and are subject to regulation by T helper cells restricted to class II MHC molecules (H-2I molecules in the mouse) (5–8). This is demonstrated by the fact that products of different class I and class II alleles vary greatly in their capacity to support the activation of CTL or T helper cells in a large number of antigenic systems (5–15). Thus, restriction specificities and class I/class II determinants are probably identical and, as immune response (Ir) gene products, regulate T cell responses. Prior research has suggested that the thymus is the site where T lymphocytes acquire their MHC-restricted recognition repertoire. Support for this concept comes from studies with $F_1 \rightarrow F_1$ radiation bone marrow chimeras that were thymectomized and engrafted with parental thymus (16, 17); $F_1 \rightarrow$ parent (P) radiation bone marrow chimeras (17); $P \rightarrow F_1$ radiation bone marrow chimeras (18–20); and fully allogeneic $A \rightarrow B$ radiation bone marrow chimeras (21). These studies suggest that it is the MHC phenotype of the thymus that determines the self–class I MHC–restricted repertoire expressed by mature CTL (16–21).

An alternate hypothesis is based on observations with athymic (nude) mice, engrafted with an allogeneic thymus. CTL from such mice failed to recognize antigens in the context of donor-type class I MHC molecules but did recognize antigen in the context of host-type class I MHC molecules (22–25), suggesting

This study was supported in part by the Foundation for Medical Research (FUNGO), which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO). Address correspondence to C. J. M. Melief, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, P. O. Box 9406, 1006 AK Amsterdam. The Netherlands. No reprints available.

[1] *Abbreviations used in this paper:* Con A, concanavalin A; CTL, cytotoxic T lymphocyte; CTLp, CTL precursor; ELISA, enzyme-linked immunosorbent assay; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; HAI, hemagglutination inhibition test; Il-2, interleukin 2; IMDM, Iscove's modified Dulbecco's medium; MHC, major histocompatibility complex; mAb, monoclonal antibody; P, parent; PBS, phosphate-buffered saline; S, Sendai virus–infected; ^{51}Cr, [^{51}Cr]sodium chromate; SPF, specific pathogen–free; TNP, trinitrophenyl.

 J. Exp. Med. © The Rockefeller University Press · 0022-1007/84/12/1752/15 $1.00
Volume 160 December 1984 1752–1766

that the thymus is not the site dictating the class I–restricted CTL repertoire. It remained unclear whether the thymus dictates the CTL precursor (CTLp) repertoire, the T helper cell repertoire, or the total T cell repertoire. Experiments on completely allogeneic thymus-engrafted nude mice and completely allogeneic radiation bone marrow chimeras suggested the existence of an extrathymic differentiation pathway for T cells restricted to class I MHC molecules in the primary CTL response to the hapten trinitrophenyl (TNP) (26–28). In contrast, only an intrathymic differentiation pathway was found for T cells restricted to class II MHC molecules (26, 29). These studies did not, however, take into account thymic influence on Ir gene phenotype, since both donor- and host-type mice were T cell responders to TNP.

We studied the H-2 Ir gene–controlled T cell responses to Sendai virus and to H-Y antigen. In C57BL/6 (B6, H-2^b) or C57BL/10 (B10, H-2^b) mice the CTL response to Sendai virus is H-$2K^b$ restricted. The H-$2K^b$ mutant mouse strain B6.C-H-2^{bm1} (bm1), is a CTL nonresponder to Sendai virus, probably due to a defect at the level of CTLp (15). In B6 or B10 mice the CTL response to H-Y is H-$2D^b$ restricted (30, 31). The H-2I-A^b mutant mouse strain B6.C-H-2^{bm12} (bm12) is a CTL nonresponder to the H-Y antigen, probably on the basis of a defect at the level of the H-2I-A-restricted T helper cell pathway (31, 32). B6 or B10 nude mice received grafts of syngeneic neonate thymus tissue (responder H-2 type for Sendai virus and H-Y) or thymus tissue from neonatal bm1 mice (Sendai CTL nonresponder type) or female bm12 mice (H-Y CTL nonresponder type). The results indicate that the thymus dictates MHC specificity and Ir gene phenotype of T cells restricted to class II MHC molecules, but not of T cells restricted to class I MHC molecules.

Materials and Methods

Animals. Normal C57BL/6, B10.A(4R), B10.A(5R), and BALB/c mice, and (mutant) bm1 and bm12 mice were bred at our laboratory under specific pathogen–free (SPF) conditions. C57BL/6 nude mice were obtained from Bomholtgard Ltd., Ry, Denmark. bm1 nude mice were bred by mating normal bm1 with C57BL/6 nude mice. The F_1 mice were mated and the F_2 nude mice were H-2-typed with a complement-dependent cytotoxicity test run on lymph node cells. For this test, we used the monoclonal antibody (mAb) K7-65, which reacts strongly with the lymphocytes of H-$2K^b$ but weakly with those of H-$2K^{bm1}$ mice. C57BL/10 nude mice were a gift from Dr. A. Singer, Bethesda, MD. The mouse strains used in this study, their H-2 haplotypes, and the structural alterations in the H-2 molecules of the mutant mouse strains are listed in Table I (33–37).

Thymus Grafts. Thymus engrafting of nude mice was performed by subcutaneous implantation of two thymuses (four lobes), obtained from donors within 24 h of birth. When these mice were used for H-Y-specific CTL responses, only female thymuses were transplanted. In >80% of the nude mice that received neonate thymus lobes, thymus tissue was demonstrable 6–8 wk after grafting. Only these mice were used for the experiments. The number of thymocytes recovered from their grafts varied from 30×10^6 to 90×10^6 cells.

Typing Reagents. Hybridoma cells producing mAb K7-65, which detects the difference between H-$2K^b$ and H-$2K^{bm1}$ (38), were a gift from Dr. U. Hämmerling, Sloan-Kettering Institute for Cancer Research, New York.

Immunofluorescence Staining and Cytofluorographic Analysis. The preparation of cells and staining procedues were as follows: 10^6 spleen cells were treated with NH_4Cl, passed over nylon wool, and incubated at 4°C for 30 min with mAb K7-65 in amounts predetermined to be saturating. They were washed twice by centrifugation, incubated at

TABLE I
H-2 Haplotypes of Mouse Strains Used in this Study

Mouse strain	Abbreviation	H-2			Structural alteration
		K	I-A	D	
C57BL/6	B6	b	b	b	—
C57BL/10	B10	b	b	b	—
B10.A(4R)	4R	k	k	b	—
B10.A(5R)	5R	b	b	d	—
BALB/c	BALB/c	d	d	d	—
B6.C-H-2^{bm1}	bm1	bm1	b	b	Glu → Ala at position 152*‡ Arg → Tyr at position 155§‡ Leu → Tyr at position 156§‡
B6.C-H-2^{bm12}	bm12	b	bm12	b	Ile → Phe at position 67‡‖ Arg → Gln at position 70‡‖ Thr → Lys at position 71‡‖

* According to Pease et al. (37).
‡ Congenic or co-isogenic with C57BL/6 (B6, H-2^b).
§ According to Nairn et al. (33).
‖ According to McIntyre et al. (36).

4°C for 30 min with saturating amounts of fluorescein isothiocyanate (FITC)-labeled goat anti-mouse antibodies (Nordic Immunological Laboratories, B.V., Tilburg, The Netherlands), washed twice more, resuspended, and analyzed for fluorescence. These procedures were performed in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin and 0.1% NaN_3. Cytofluorographic analysis was performed using a cytofluorograph (FC200; Ortho Diagnostics, Westwood, MA) (39). Fluorescence intensity is expressed in arbitrary units (μG), as defined earlier (39).

Preparation of Interleukin 2 (IL-2). Spleen cells from Wistar rats were cultured for 22 h at a density of 5×10^6 cells/ml, 3×10^6 cells/cm^2 with 5 ng/ml phorbol myristate acetate in Iscove's modified Dulbecco's medium (IMDM) (Gibco Laboratories, Grand Island, NY) supplemented with 5% fetal calf serum (FCS) (Gibco Laboratories), 100 IU/ml penicillin, 100 μg/ml streptomycin, and 5×10^{-5} M 2-mercaptoethanol. Cells were then pulsed for 2 h with 5 μg/ml concanavalin A (Con A), washed three times, and cultured in lectin-free medium for another 24 h, after which the supernatants were harvested, filtered, and tested for their content of IL-2 by measuring [^{3}H]thymidine incorporation in an IL-2-dependent T cell line (40). These supernatants were used at a final concentration that was optimal for the particular preparation, usually 25% (vol/vol).

Sendai Virus. Infectious Sendai virus, lot 40340086, was obtained from Flow Laboratories (McLean, VA) and was stored at −70°C. The virus had been propagated in pathogen-free eggs. The titer was 10^4 hemagglutination units (HAU)/ml.

Immunization. Normal mice, nude mice, and thymus-engrafted nude mice that had received thymuses 8 wk earlier, were primed by one intraperitoneal injection of 10^2 HAU infectious Sendai virus. Female mice (normal or thymus-engrafted nudes) were primed with H-Y antigen by a single intraperitoneal injection of 10×10^6 syngeneic male spleen cells.

In Vitro Generation of Sendai-specific CTL. According to the method of De Waal et al. (15), 30×10^6 to 70×10^6 responder spleen cells from 10-wk-old mice (primed in vivo 3 wk earlier) were cocultured with the same number of irradiated (2,000 rad) spleen cells coated with Sendai virus (stimulator cells). This was done in 50 ml culture medium for 5 d at 37°C in humidified air with 5% CO_2. For the preparation of stimulator cells, 10^8 NH_4Cl-treated spleen cells were incubated in 2 ml culture medium with 6×10^2 HAU infectious Sendai virus for 1 h at 37°C. They were then washed three times with culture medium consisting of IMDM supplemented with 10% pooled human serum, penicillin (100 IU/ml), streptomycin (100 μg/ml), and 2-mercaptoethanol (2×10^{-5} M).

In Vitro Generation of H-Y-specific CTL. Responder spleen cells (30×10^6 to 60×10^6) from 10-wk-old female mice (primed in vivo 3 wk earlier) were cocultured with the same number of irradiated (2,000 rad) male spleen cells. This was done in 50 ml culture medium for 5 d at 37°C in humidified air with 5% CO_2.

Measurement of CTL Effector Activity. Varying numbers of effector cells were added to 1.5×10^4 [^{51}Cr]sodium chromate (^{51}Cr)-labeled target cells in 0.2 ml IMDM supplemented with 10% FCS in wells of round-bottomed microtiter plates. These were incubated for 4 h (in the Sendai system) or 3 h (in the case of H-Y), at 37°C in humidified air with 5% CO_2. After incubation, the supernatant was collected with the Titertek Supernatant Collection System (Flow Laboratories). As target cells, we used Con A–induced (2.5 μg/ml) lymphoblasts (2–3 d old) in the Sendai system, and LPS-induced (30 μg/ml) lymphoblasts (3–4 d old) in the H-Y system.

For Sendai virus modification, 10^7 target cells were incubated with 3×10^2 HAU infectious Sendai virus for 1 h at 37°C. The percentage specific ^{51}Cr release was calculated by the formula: Percent specific lysis = (cpm experimental − background ^{51}Cr release)/(cpm 5% saponin release − background ^{51}Cr release). Background ^{51}Cr release was the release seen in the presence of responder spleen cells cocultured with irradiated syngeneic spleen cells. Background ^{51}Cr release was always <22%. In the Sendai system no difference was observed between virus-infected and noninfected target cells. The standard error of triplicate cultures was always <3% specific ^{51}Cr release.

Determination of Sendai-specific Antibody Titers. The titer of Sendai-specific antibodies in mouse sera was determined with a hemagglutination inhibition test (41) and an enzyme-linked immunosorbent assay (ELISA). The ELISA was performed by coating microtiter plates with 0.2 ml/well Sendai virus (0.005 mg protein/ml) by overnight incubation at 4°C. After washing in PBS with 0.1% Tween 20, 0.05 ml diluted test serum was added. The sera were diluted in PBS/Tween containing 10% FCS. Subsequently, the plates were incubated for 1 h at 37°C and were then washed three times in PBS/Tween. Finally, 0.05 ml of peroxidase-labeled rabbit anti-mouse IgG (Miles Laboratories, Elkhart, IN), at a 1:1000 dilution in PBS/Tween/FCS, was added. After 1 h at 37°C, the plates were washed three times of PBS/Tween, and the diluted substrate (orthophenylenediamine/H_2O_2) was added. After 30 min, the reaction was stopped by adding 0.1 ml 3 N H_2SO_4. The optical density of the reaction, as a measure of the amount of specific IgG, was determined by spectrophotometry (Titertek Mullisham; Flow Laboratories).

Results

H-2K^b Mutant bm1 Neonate Thymuses Subcutaneously Grafted Into H-2K^b Nude Mice Result in a Mature H-2K^b Surface Phenotype of Nude Host Splenic T Lymphocytes. The H-2 phenotype of splenic T lymphocytes from thymus-engrafted nude mice was determined. Fig. 1, *A* and *B* illustrate that, 8 wk after subcutaneous grafting of a syngeneic neonatal thymus into an H-2^b nude mouse, the low H-2K^b expression of the nude T cells had changed to the high H-2K^b expression characteristic of normal (mature) H-2K^b T cells (Fig. 1 *C*). Fig. 1 *E* illustrates the H-2 expression of the splenic T cells from H-2^b nude mice that are subcutaneously implanted with thymus tissue from neonatal H-2K^b mutant bm1 mice. It can be seen that, in these thymus-engrafted nude mice, the T cells in the spleen are of host origin, since they express the mature H-2K^b phenotype of the H-2^b nude host, as detected by the K7-65 mAb (Fig. 1 *C*), and not the mutant H-2K^{bm1} phenotype of the thymus graft (Fig. 1 *D*).

Transplantation of Neonate Thymus Results in Differentiation of CTLp in Athymic Nude Mice. Athymic nude mice do not mount a CTL response to allogeneic cells in vitro, even in the presence of exogenous Il-2 (Fig. 2). However, transplantation of a neonatal B6 thymus into a B10 nude mouse resulted in the

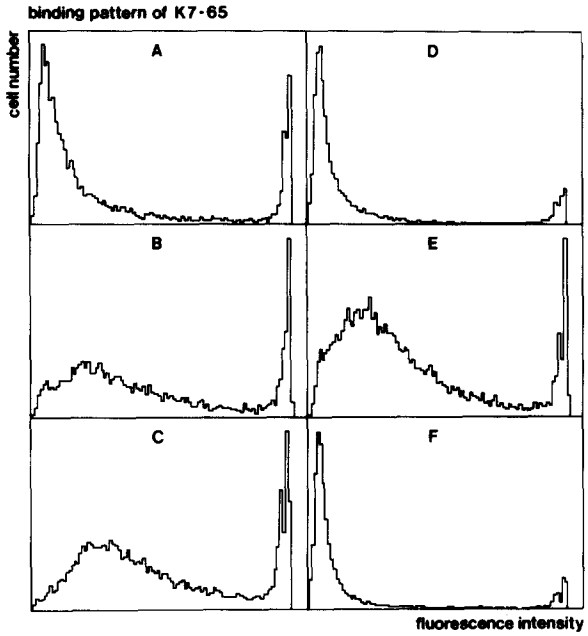

FIGURE 1. Cytofluorographic staining profiles with monoclonal antibody K7-65, directed against H-2K^b and FITC-labeled goat anti-mouse antibodies. (*A*) Spleen cells of H-2^b nude mice, (*B*) spleen cells of H-2^b nude mice engrafted with B6 thymus, (*C*) normal B6 (H-2^b) spleen cells, (*D*) normal bm1 spleen cells, (*E*) spleen cells of H-2^b nude mice engrafted with bm1 thymus, and (*F*) spleen cells from *A–E* stained only with FITC-labeled goat anti-mouse antibodies.

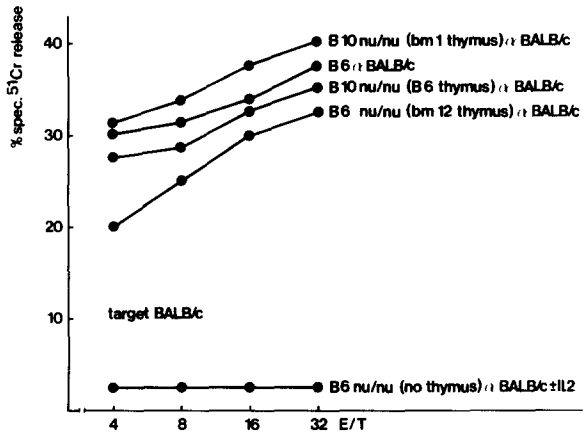

FIGURE 2. Allogeneic CTL generated in B6 and H-2^b nude mice engrafted with thymus tissue as indicated in brackets, when stimulated with irradiated (2,500 rad) BALB/c spleen cells and tested against BALB/c target cells.

generation of a strong primary CTL response to allogeneic BALB/c cells. The same result was found after transplantation of thymus tissue from (H-2K^b mutant) bm1 or (H-2I-A^b mutant) bm12 neonates into B10 nude mice. These findings demonstrate that the thymus is vital for the generation of allospecific CTL.

Transplantation of Thymuses from (H-2K^b Mutant) bm1 Neonates Into H-2^b Nude

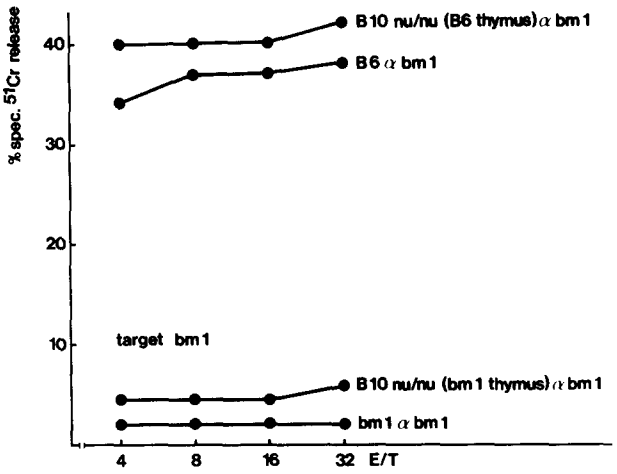

FIGURE 3. Allogeneic CTL generated in B6, bm1, and B10 nude mice, engrafted with thymus tissue as indicated in brackets, when stimulated with irradiated (2,500 rad) bm1 spleen cells and tested against bm1 target cells.

Mice Induces Tolerance of bm1. C57BL/10 nudes that received thymic grafts from B6 neonates generated anti-bm1 CTL responses (Fig. 3). In contrast, B10 nudes that received thymuses from bm1 neonates did not mount CTL responses against bm1 cells, indicating that B10 nude mice engrafted with bm1 thymuses became tolerant of bm1.

Thymus Does Not Dictate MHC Specificity and Ir Gene Phenotype of T Cells Restricted to Class I MHC Molecules. The same mice used for their capacity to generate allogeneic CTL responses (Fig. 2) were simultaneously tested for their ability to mount a Sendai virus–specific CTL response. Whether or not exogenous Il-2 is added, athymic B10 nude mice do not generate Sendai virus–specific CTL responses after in vivo priming followed by restimulation in vitro (Fig. 4).

Engraftment of a B6 neonate thymus into a B10 nude mouse allowed the generation of H-2K^b-restricted Sendai-specific CTL since only Sendai-coated H-2K^b-bearing targets were lysed (Fig. 4, Table II), and not Sendai-coated bm1 targets. In addition, engraftment of mutant bm1-type thymus into a B10 nude mouse allowed maturation of Sendai virus–specific CTL that were H-2K^b restricted (Fig. 4, Table II). Similar results were obtained in four independent experiments. In the reverse situation (Table III), bm1 nude mice engrafted with either syngeneic neonatal bm1 thymuses (Sendai CTL nonresponders) or neonatal B6 thymuses (Sendai CTL responders) remained Sendai virus–specific CTL nonresponders. Allogeneic CTL responsiveness, however, was restored. Notably, bm1 nude mice transplanted with B6 thymuses were nonresponsive to B6 cells (Table III). Similar results were obtained in three independent experiments. Together, these results demonstrate that the thymus does not alter the MHC specificity or Ir gene phenotype of T cells restricted to class I MHC molecules.

Thymus Dictates MHC Specificity and Ir Gene Phenotype of T Cells Restricted to Class II MHC Molecules. To determine thymic influence on the Ir gene phenotype of class II–restricted T cells, we studied the CTL response against H-Y antigen. The H-2I-A^b (class II) mutant bm12 strain is a CTL nonresponder for

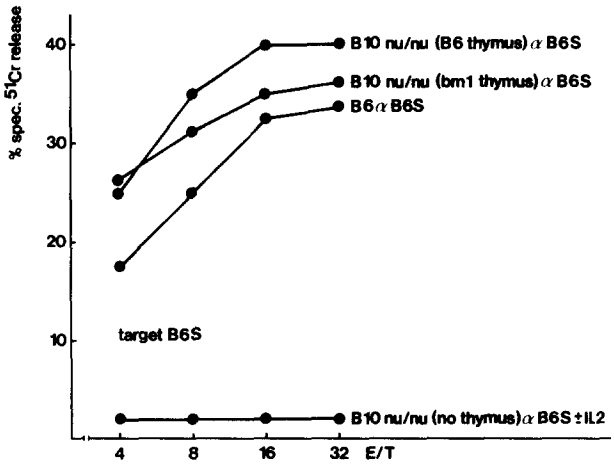

FIGURE 4. Sendai virus–specific CTL generated in B6 and B10 nude mice, engrafted with thymus tissue as indicated in brackets, when primed in vivo and restimulated in vitro with Sendai-infected irradiated (2,500 rad) B6 spleen cells and tested against Sendai virus–infected B6 target cells. Lysis of non–virus-infected B6 target cells was <5% specific lysis.

TABLE II

CTL Response Against Sendai Virus: Effect of Responder-Nonresponder Neonate Thymus Transplantation Into Responder Nude Host Mice

Responder Cells*	Stimulator cells‡	Target cells§					
		bm1S	bm1	4RS‖	4R	5RS	5R
B10 nude (B6 thymus)	B6S	0¶	2	1	2	38	4
B10 nude (bm1 thymus)	B6S	−4	−2	2	1	32	3
B6	B6S	8	4	4	2	31	2
bm1	bm1S	9	6	5	3	6	3

* Spleen cells from Sendai-primed mice, engrafted with thymus tissue as indicated in parentheses.
‡ Sendai virus–infected (S) B6 or bm1 spleen cells (irradiated with 2,500 rad).
§ 1.5×10^4 Con A blasts. S, Sendai virus–infected.
‖ 4R Sendai virus–infected cells are always killed by syngeneic anti-Sendai effector cells (not shown).
¶ Percentage specific ^{51}Cr release at effector-to-target cell ratio of 32:1 (means of triplicate determinations).

this antigen. Whether or not IL-2 is added, athymic female B6 nude mice do not generate an H-Y-specific CTL response after priming in vivo and restimulation in vitro. Engraftment of female B6 thymus into a female B6 nude allowed generation of H-Y-specific CTL. Since, in H-2^b mice, this CTL response is H-$2D^b$ restricted (30, 31), both male B6 and bm12 targets were lysed.

Engraftment of female bm12 thymuses into female B6 nude mice did not allow maturation of H-Y-specific CTL (Fig. 5). Spleen cells of the same mouse were perfectly capable of mounting an allogeneic CTL response against BALB/c (Fig. 2). Similar results were obtained in three independent experiments. These results indicate that the thymus does dictate the Ir gene phenotype of T cells restricted to class II MHC molecules.

Lack of H-Y-specific CTL Response in Female B6 Nude Mice Receiving bm12 Thymus Tissue Is Reversed by Engraftment of B6 Thymus. A female B6 nude mouse

TABLE III

CTL Response Against Sendai Virus: Effect of Responder-Nonresponder Neonate Thymus Transplantation Into Nonresponder Nude Host Mice

Responder cells*	Stimulator cells‡	Target cells§				
		B6S	B6	bm1S	bm1	BALB/c
bm1 nude (B6 thymus)	B6S	14‖	13	1	0	7
bm1 nude (B6 thymus)	bm1S	6	2	6	1	4
bm1 nude (B6 thymus)	BALB/c	0	0	0	0	72
bm1 nude (bm1 thymus)	B6S	76	82	2	0	29
bm1 nude (bm1 thymus)	bm1S	0	0	0	0	0
bm1 nude (bm1 thymus)	BALB/c	8	9	0	0	76
B6	B6S	40	9	12	9	8
bm1	bm1S	8	4	10	5	6

* Spleen cells from Sendai-primed mice, engrafted with thymus tissue as indicated in brackets.
‡ Uninfected BALB/c and Sendai virus–infected (S) B6, bm1 spleen cells (irradiated with 2,500 rad).
§ 1.5×10^4 Con A blasts.
‖ Percentage specific ^{51}Cr release at effector-to-target cell ratio of 32:1 (means of triplicate determinations).

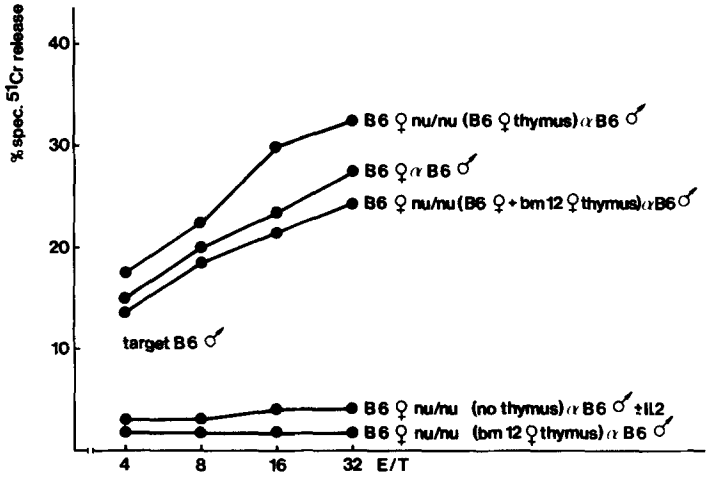

FIGURE 5. H-Y-specific CTL generated in B6 and B6 nude mice, engrafted with thymus tissue as indicated in brackets, when primed in vivo and restimulated in vitro with irradiated (2,500 rad) male B6 spleen cells and tested against male B6 target cells. Lysis of female B6 target cells was <5% specific lysis.

received thymus tissue from a female B6 (H-Y CTL responder) neonate on one side and, simultaneously, on the other side, received a thymic graft from a neonatal female bm12 (H-Y CTL nonresponder) mouse. Thymus tissue of similar size was demonstrable on both sides 8 wk after grafting. Spleen cells of these mice generated a strong CTL response against H-Y antigen in two independent experiments (Fig. 5). This indicates that the lack of an H-Y-specific CTL response in female B6 nude mice receiving thymus tissue from female bm12 neonates is probably not caused by a suppressive effect from the nonresponder type thymus.

Thymus Restores Anti-Sendai Humoral Antibody Response in Athymic Nude Mice. The anti-Sendai antibody responses of nude mice primed in vivo but

TABLE IV

Titer of Sendai Virus–specific Antibodies in Sera From Sendai Virus–immunized Mice

Serum source		Primed in vivo with Sendai virus	Sendai-specific antibody titer	
Mouse strain	Thymus graft		ELISA	HAI*
B10 nude	—	+	<50	0
B10 nude	B6 thymus	+	2,400	10
B10 nude	bm1 thymus	+	3,200	10
B6		+	3,800	40
bm1		+	3,200	10
B10 nude	—	—	<50	0
B6		—	<50	0
bm1		—	<50	0

* Hemagglutination inhibition test

receiving no thymus grafts were compared with the responses of nude mice that received neonatal thymus grafts. The results shown in Table IV indicate that, after immunization with Sendai virus, athymic B10 nudes did not form Sendai-specific antibodies. However, engraftment with thymuses from either B6 or bm1 neonates restored the antibody response. This indicates that the T helper cell pathway for specific antiviral antibody production in these mice is restored as a result of thymus engraftment.

Discussion

The thymus dictates the MHC specificity and Ir gene phenotype of T cells restricted to class II MHC molecules but not of T cells restricted to class I MHC molecules. To demonstrate this we took advantage of the availability of class I (bm1) and class II (bm12) H-2^b mutant mouse strain. The H-$2K^b$ molecule of the mutant bm1 strain differs by three amino acids from the H-$2K^b$ molecule of B6 (33, 37). As a result, it is a CTL nonresponder to Sendai virus (15). The I-A^b_β molecule of the bm12 mutant also differs by three amino acids (36) from the B6 product, making bm12 a CTL nonresponder to H-Y antigen (31). Nonresponsiveness in the class I mutant, bm1, is probably determined at the level of the CTLp repertoire (unpublished observations); whereas, in the class II mutant, bm12, nonresponsiveness lies at the level of the T helper cell repertoire (31).

The thymus provides a necessary environment for the maturation of all CTLp, and dictates the class II MHC restriction of T cells but not the class I MHC restriction. These conclusions derive from the following observations: (*a*) Athymic H-2^b nude mice engrafted with either B6 or bm1 thymuses are CTL responders to Sendai virus, while nonengrafted nude mice do not respond. In both cases the Sendai-specific CTL response is H-$2K^b$ restricted. (*b*) In contrast to the effect of introducing a B6 thymus, engraftment of a bm12 thymus into a nude H-2^b mouse did not lead to the ability to generate H-Y-specific CTL responses. (*c*) All thymus grafts resulted in a mature H-2^b splenic T cell surface phenotype in the nude hosts, and restored the CTL response to alloantigens.

The dichotomy of thymic influence on the class I and II MHC–restricted T

cell repertoires is also confirmed by experiments showing that CTL responses in athymic nude mice can be detected after adding IL-2 in vitro or in vivo (42–44). Antigen-driven T cell differentiation, which does not occur in nude mice, might account for the difference in CTL repertoire between nudes and normal mice (43). Another important observation is the existence of an extrathymic pathway for differentiation of class I MHC–restricted anti-TNP CTLp, but not of class II MHC–restricted T helper cells (26–29). In those studies, an additional intrathymic pathway for CTLp differentiation was identified (26–28). Our experiments do not exclude the possibility of an intrathymic CTLp differentiation pathway because Sendai-specific CTL were generated from the thymuses of B6 nudes engrafted with nonresponder type bm1 thymuses, and because the H-$2K^{bm1}$ molecule of the nonresponder type thymus cannot serve as a restriction element for Sendai-specific CTL (15).

Selection of the T cell repertoire in the thymus may depend on the class I and class II MHC molecule expression in thymic cells potentially involved in the selection process. It has been postulated that thymic selection is based on the T cells' affinity for self-MHC (45). Interaction of precursor T cells with H-2 Ia-bearing cells appears to be necessary for appropriate maturation of T helper cells. This, especially, suggests that thymic selection concerns class II MHC specifically (46, 47). Indeed, the presence of class II (but not class I) MHC antigens on the cortical epithelium (48, 49) might suggest selection for class II–reactive cells. Conversely, the presence of both class I and II MHC antigens in the medulla (42, 43) might imply that cells of either MHC restriction type differentiate there. Recently (50, 51), it has been shown that thymectomized, bone marrow–reconstituted radiation chimeras develop CTLp that can respond to antigen only if Con A supernatant is added to the culture, indicating that a T helper cell defect is the cause of nonresponsiveness in these chimeras.

Although the thymus does not dictate the MHC specificity and Ir gene phenotype of class I MHC–restricted CTL, the thymus is necessary for final maturation of CTLp. Apparently, extrathymic determination of the CTLp repertoire occurs, be it pre- or postthymic passage. Mechanistically, it is of interest that clonal abortion of bone marrow T cell precursors for certain antigens or allogeneic H-2 determinants can lead to specific T cell nonresponsiveness, suggesting that T cell receptor expression occurs prethymically (52, 53).

Because of the existence of an extrathymic pathway for the generation of the class I but not class II MHC–restricted T cell repertoire, class II MHC–restricted T cell precursors are expected to be less differentiated at the moment of migration into the thymus than class I MHC–restricted T cells. The thymus apparently exerts a major influence on T cells restricted to class II molecules, including repertoire formation, whereas its role with respect to class I MHC–restricted T cells is limited to a final maturation step without gross repertoire changes.

In experiments with allogeneic thymus grafts, however, some repertoire changes are expected for class I–restricted CTL; a result of the development of tolerance to the allogeneic thymus. Apparently, such a change in the T cell repertoire was not sufficient to prevent bm1 thymus-engrafted B10 nudes from

responding to Sendai virus. The specificity of Sendai-specific K^b-restricted CTL, then, does not overlap with the B6 anti-bm1 allo-repertoire.

Our data rule out the possibility that a thymic remnant (which exists in nude mice, though not populated with T cells; 54), can account for the "extrathymic" host-restricted CTL repertoire observed in the H-2^b nude mice that have received bm1 thymus tissue. This possibility can be excluded because H-2^b nude mice engrafted with bm12 thymuses were CTL nonresponders to H-Y, even though they had a thymic remnant of responder type.

The failure of bm12 thymuses to confer H-Y-specific T cell responsiveness when grafted into nude B6 recipients could be due to several mechanisms that have been postulated for Ir gene–controlled nonresponsiveness. These mechanisms include: (*a*) failure to positively select class II MHC–restricted T helper cells; (*b*) creation of deletions from the T helper cell repertoire; (*c*) inappropriate antigen presentation; and (*d*) generation of specific suppressor T cells. The last possibility is unlikely since transplantation of both B6 and bm12 thymuses into a B6 nude host restored the capacity to respond against H-Y. Defective antigen presentation remains a possibility, although it has been shown (31), for the H-Y system, that bm12 (nonresponder type) antigen-presenting cells actually can present the antigen, at least in secondary in vitro stimulation.

So far we have discussed situations in which nonresponder type thymuses were grafted into responder type nude mice. The reverse situation (responder type thymus into nonresponder type nude host) was also tested in the case of class I nonresponders. Nude bm1 mice engrafted with B6 thymus tissue were Sendai virus CTL nonresponders, despite the presence of a responder type thymus in which these CTLp could have learned their self-H-2^b specificity and Ir gene phenotype. This outcome is not so surprising, however, since the bm1 nude host lacks an appropriate class I MHC restriction element for stimulation of a Sendai virus–specific CTL response (15). A more definitive experiment would be to transfer the CTLp of these mice into irradiated thymectomized adult B6 mice and evaluate whether they can be educated against Sendai virus in the context of H-$2K^b$. Furthermore, it will be of interest to explore whether the class I/class II dichotomy with respect to the role of the thymus also holds in chimeras, using the same class I/class II nonresponder H-2 mutants. Although chimera experiments may be criticized, because of the very poor survival of the chimeras under conventional animal housing conditions, it is now possible to raise healthy long-lived chimeras under SPF conditions (55).

In conclusion, extrathymic mechanisms play a significant role in determining the self-specificity and Ir gene phenotype of T cells restricted to class I MHC molecules. In contrast, only intrathymic mechanisms appear to play a role in determining the self-specificity and Ir gene phenotype of T cells restricted to class II MHC molecules.

Summary

Athymic H-2^b nude mice received grafts from C57BL/6 (Sendai virus and H-Y antigen cytotoxic T lymphocyte [CTL] responder type), bm1 (H-$2K^b$ mutant, Sendai CTL nonresponder type), or bm12 (H-2I-A mutant, H-Y CTL nonresponder type) neonates. In observations of the CTL response to H-Y, both

recipients and thymus donors were female. All types of thymus engraftment resulted in mature H-2^b splenic T lymphocyte surface phenotype in nude hosts. T cell immunocompetence (as measured by major histocompatibility complex [MHC] CTL responses to allogeneic cells) was restored, and induced nonresponsiveness to the MHC determinants of the engrafted thymus in the nude host. The CTL reaction to Sendai virus in both responder type C57BL/6 and nonresponder type bm1 neonatal thymuses allowed maturation of Sendai-specific, H-$2K^b$-restricted CTL. For the CTL reaction to H-Y, only responder type C57BL/6 thymuses restored the CTL response, whereas this was not achieved with thymuses from nonresponder type bm12 neonatal females. Results of double thymus (B6 and bm12) engraftment excluded the possibility that this latter effect was caused by suppression.

In addition, athymic bm1 mice were engrafted with thymuses from either B6 (Sendai CTL responder type) or syngeneic bm1 neonates (Sendai CTL nonresponder type). Again, both types of neonate thymuses restored T cell competence as measured by MHC/CTL responses to allogeneic cells. However, neither responder B6 nor nonresponder bm1 neonate thymus grafts allowed maturation of Sendai-specific CTL. In conclusion, the thymus dictates MHC specificity and immune response gene phenotype of T cells restricted to class II MHC molecules but not of T cells restricted to class I MHC molecules.

We acknowledge J. van de Weg for thymus engrafting, all the animal technicians for maintaining the mice, T. Leupers for H-2 typing, and J. T. M. van der Logt (ICLAS, Nijmegen) for determination of Sendai-specific antibody titers. We thank A. M. Kruisbeek and A. Singer for stimulating discussions that led us to do these experiments. We also thank C. J. Lucas, C. P. Engelfriet, P. Iványi, M. J. Bevan, and G. M. Shearer for reviewing our manuscript.

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