Brief Definitive Report

SENDAI VIRUS-SPECIFIC, H-2-RESTRICTED CYTOTOXIC T LYMPHOCYTE RESPONSES OF NUDE MICE GRAFTED WITH ALLOGENEIC OR SEMI-ALLOGENEIC THYMUS GLANDS*

By JEFFREY P. LAKE, MARION E. ANDREW, CARL W. PIERCE, and THOMAS J. BRACIALE‡

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis; and the Departments of Pathology and Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri 63110

The thymus has been implicated as the site where T cells acquire the capacity to recognize major histocompatibility complex (MHC) gene products. The strongest support for this concept has come from studies with irradiation bone marrow chimeric mice (1, 2). Recently an alternative model system, the thymus gland-grafted athymic (nude) mouse, has been used to test this concept for cytotoxic T lymphocytes (CTL) (3, 4). To date, inconsistent results have been obtained. On one hand, (A × B)F_1 nude mice implanted with thymus glands from either parental strain developed virus-specific CTL that lysed exclusively virus-infected target cells bearing MHC antigens identical to the thymus donor (3, 4), a result consistent with data in the chimera system. However, in sharp contrast, cells from nude mouse (A) maturing in an MHC incompatible (B) thymus graft or in a semiallogeneic (A × B)F_1 thymus graft did not acquire the ability to recognize (B) MHC antigens as self (4).

We have examined the in vitro secondary Sendai virus-specific CTL responses of spleen cells from a variety of thymus-grafted nude mice and observed that: (*a*) spleen cells from BALB/c (H-2^d) nude mice bearing allogeneic C57BL/10 (H-2^b) thymus glands developed Sendai virus-specific CTL that lysed Sendai virus-infected BALB/c, but not C57BL/10 target cells; and (*b*) spleen cells from (C57BL/6 × BALB/c)F_1 nude mice bearing thymus glands from either parent developed Sendai virus-specific CTL that preferentially lysed Sendai virus-infected target cells of the thymus donor haplotype, but also lysed Sendai virus-infected target cells of the other parental haplotype. This degree of preferential lysis varied among individual thymus-grafted nude mice. Implications of these results for the process of MHC recognition by T cells are discussed.

Materials and Methods

Virus. Sendai virus obtained from Dr. Peter Cooper (Australian National University, Canberra City, Australia) was grown in the allantoic cavity of 10-d-old chick embryos and

* Supported by grants AI-15353 and AI-15608 from the U. S. Public Health Service (National Institute of Allergy and Infectious Diseases) and by grant IN-36-S from the American Cancer Society.

‡ Research supported by Brown and Williamson Tobacco Corporation, Philip Morris Incorporated, R. J. Reynolds Tobacco Company, and the United States Tobacco Company.

stored at −70°C until used. Virus was inactivated by exposure of 10^5 hemagglutinating units/ml to a 25-W ultraviolet (UV) lamp at 20 cm for 10 min with constant agitation. No residual viral infectivity was detected after inoculation into embryonated eggs.

Mice and Immunizations. Conventional BALB/c (H-2^d), C57BL/6 (H-2^b), C57BL/10 (H-2^b), and (BALB/C × C57BL/10)F_1 (H-2^d × H-2^b) mice were bred and maintained in the Animal Facility of the Jewish Hospital of St. Louis, St. Louis, Mo. BALB/c nu/nu breeding stock was kindly provided by Dr. Norman Reed, Montana State University and have undergone 20 generations of inbreeding (10 backcross generations) into the BALB/c strain using cross-intercross mating. (C57BL/6 × BALB/c)F_1 nu/nu mice were produced by mating male BALB/c nu/nu with female C57BL/6 +/nu mice obtained from G. L. Bomholtgaard Ltd., Ry, Denmark.

Mice were immunized by a single intravenous inoculation of infectious Sendai virus (1×10^7–5×10^7 infectious doses of virus). All mice survived this inoculation without evidence of generalized infection and were used as donors of primed CTL precursors 3–6 wk after innoculation.

Thymus Grafting. Two or three intact thymus lobes from neonatal (<24 h) mice were implanted under the capsule of one kidney of 8- to 12-wk-old nude mice as described by Metcalf (5). Thymus gland-grafted nude mice were used 6–8 mo after grafting.

Generation and Assay of In Vitro Secondary Sendai Virus-specific CTL. CTL were generated in vitro by incubating 25×10^6–50×10^6 spleen cells from individual primed, nude, thymus-grafted nude, or conventional mice for 5 d with appropriate stimulator cells (responder: stimulator ratio of 10:1) as described in detail previously (6). Stimulator cells were irradiated (2,000 rad) spleen cells that had been treated with UV-inactivated Sendai virus at 1,000 hemagglutinating units/10^6 nucleated cells. CTL responses were assayed as previously detailed (6). Lymphoblasts, generated by incubation of normal spleen cells for 48 h with 2 μg/ml Concanavalin A (Miles Laboratories, Inc., Elkhart, Ind.) were used as target cells. Lymphoblasts were simultaneously infected with Sendai virus and labeled with ^{51}Cr by incubation for 1.5 h at 37°C in serum-free medium with 10 infectious units of Sendai virus/cell and 50–150 μCi of ^{51}Cr as sodium chromate (New England Nuclear, Boston, Mass.)/10^6 cells.

Results and Discussion

Adult BALB/c (H-2^d) nude mice were implanted with neonatal syngeneic BALB/c or allogeneic C57BL/10 (H-2^b) thymus glands and 6–8 mo later were primed with infectious Sendai virus. 3 wk after priming, spleen cells from individual mice were cultured with irradiated, Sendai virus-treated BALB/c or C57BL/10 stimulator cells. Spleen cells from BALB/c nude mice grafted with BALB/c thymus glands (BALB/c Thy → BALB/c Nu) developed a CTL response after stimulation with Sendai virus-treated syngeneic stimulator cells (Table I, group 1). Spleen cells from BALB/c nude mice grafted with C57BL/10 thymus glands (B10 Thy → BALB/c Nu) also developed a CTL response when stimulated with Sendai virus-treated BALB/c stimulator cells (group 3). Both responses were comparable in magnitude to that of spleen cells from conventional BALB/c mice (group 6), and were directed exclusively to Sendai virus-infected BALB/c target cells with no detectable activity on infected or uninfected C57BL/10 target cells. Stimulation of B10 Thy → BALB/c Nu spleen cells with Sendai virus-treated C57BL/10 stimulator cells yielded no detectable CTL response on infected or uninfected C57BL/10 target cells (group 4). By contrast, stimulation of spleen cells from conventional BALB/c or BALB/c Thy → BALB/c Nu mice with Sendai virus-treated C57BL/10 stimulator cells resulted in responses against C57BL/10 alloantigens; both Sendai virus-infected and uninfected C57BL/10 target cells were lysed (groups 2 and 7). However, spleen cells from all BALB/c mice stimulated with Sendai virus-treated C57BL/10 stimulator cells (groups 2, 4, and 7) also had activity specific for Sendai virus-infected BALB/c target cells. This unanticipated

TABLE I

*Sendai Virus-specific CTL Responses of Nude mice with Allogeneic Thymus Grafts**

Group	Responder spleen cells	Sendai virus-treated stimulator cells	Effector:target cell ratio‡	Percent ^{51}Cr release§			
				S-BALB/c‖	BALB/c	S-C57BL/10	C57BL/10
1	BALB/c Thy → BALB/c Nu	BALB/c	1	62	1	6	0
			5	93	4	9	2
			10	93	5	15	6
2	BALB/c Thy → BALB/c Nu	C57BL/10	1	73	0	9	0
			5	93	6	16	13
			10	100	7	24	25
3	B10 Thy → BALB/c Nu	BALB/c	1	38	0	1	0
			5	61	0	3	0
			10	77	0	1	0
4	B10 Thy → BALB/c Nu	C57BL/10	1	29	0	0	0
			5	60	0	0	0
			10	65	0	0	0
5	BALB/c Nu	BALB/c	30	0	0	NT¶	NT
6	BALB/c	BALB/c	1	35	0	2	0
			5	68	2	1	0
			10	82	4	5	0
7	BALB/c	C57BL/10	1	45	0	4	4
			5	74	6	18	10
			10	91	6	30	19
8	C57BL/10	BALB/c	1	17	5	17	0
			5	39	24	44	0
			10	46	39	60	3
9	C57BL/10	C57BL/10	1	5	0	19	0
			5	7	2	45	4
			10	9	5	63	2

* Spleen cells from the indicated individual conventional, nude, or thymus gland-grafted nude mice immunized with Sendai virus were stimulated in vitro with Sendai virus-treated BALB/c or C57BL/10 stimulator cells under conditions described in Materials and Methods. Spleen cells from four thymus gland-grafted BALB/c nude mice of each type were examined with comparable results; representative data are shown.

‡ 2×10^4 ^{51}Cr-labeled lymphoblast target cells/well.

§ Values are the mean percent specific ^{51}Cr released from three replicate wells with spontaneous release subtracted. SE were <5% of the mean in all cases and are omitted.

‖ S, Sendai virus-infected lymphoblast targets of the indicated strain. Spontaneous ^{51}Cr released from the target cells ranged from 25 to 35%.

¶ NT, not tested.

CTL activity appeared to be stimulated as the result of transfer of Sendai viral antigens from the designated stimulator cells to cells in the responder cell population. Spleen cells from sendai virus-primed, but ungrafted, BALB/c nude mice failed to develop a detectable CTL response (group 5).

The failure to demonstrate a Sendai virus-specific, H-2^b-restricted CTL response by spleen cells from B10 Thy → BALB/c Nu mice (group 4) could be a result of: (*a*) the inability of an allogeneic (H-2^b) thymus to provide the appropriate environment for maturation of H-2^d host stem cells to allow recognition of virus in association with H-2^b stimulator cells; (*b*) the lack of H-2^b antigen-presenting cells in the environment where priming occured; or (*c*) potential interference with development of a weak sendai virus-specific, H-2^b-restricted CTL response by a strong Sendai virus-specific, H-2^d-restricted CTL response because of antigen carry-over. However, the critical

observation that spleen cells from B10 Thy → BALB/c Nu mice were capable of developing a potent Sendai virus-specific CTL response restricted to the host (H-2^d) haplotype (group 3) was clearly not influenced by these factors. This appears to contradict the theory first proposed by Zinkernagel (1) that T cells are selected in the thymus for recognition of conventional antigen in association with MHC antigens displayed on the thymus epithelium. However, the experiments that led to this theory used, for the most part, chimeric mice in which the thymus, stem cells, and peripheral lymphoreticular cells were at least semisyngeneic. It is possible that when the thymus and all other lymphoreticular elements are MHC incompatible, normal T cell maturation cannot occur (1). It was necessary to examine the role of the thymus in MHC restricted self-recognition in F_1 nude mice implanted with parental thymus glands where the grafted thymus and the host were partially MHC compatible.

Adult (C57BL/6 × BALB/c)F_1 nude mice were implanted with neonatal BALB/c or C57BL/6 thymus glands, immunized with Sendai virus 6 mos later, and spleen cells cultured with UV-Sendai virus-treated (BALB/c × C57BL/10)F_1 stimulator cells 3 wk later. The results in Table II represent the range of Sendai virus-specific CTL responses observed. Spleen cells from the majority of (C57BL/6 × BALB/c)F_1 nude mice tested exhibited a preference for target cells of the haplotype of the implanted thymus (groups 1, 2, and 3). These CTL also had detectable activity, but, to a lesser degree, on infected target cells of the parental H-2 haplotype not represented in the thymus. Furthermore, spleen cells from two of eight mice tested had essentially equivalent activity on infected target cells of both parental haplotypes (group 5). Although the reason(s) for this variation among experimental animals is not clear,

TABLE II

*Sendai Virus-specific CTL Response of F_1 Nude Mice with Parental Thymus Gland Grafts**

Experiment	Group	Responder spleen cells	Sendai virus-treated stimulator cells	Effector cell:target cell ratio‡	Percent ^{51}Cr release‡			
					S-BALB/c‡	BALB/c	S-C57BL/10	C57BL/10
1	1	BALB/c Thy → (C57BL/6 × BALB/c)F_1 Nu	(BALB/c × C57BL/10)	1	20	0	0	5
				5	50	0	6	1
				10	71	0	13	3
	2	B6 Thy → (C57BL/6 × BALB/c)F_1 Nu	(BALB/c × C57BL/10)	1	1	0	15	4
				5	8	0	48	3
				10	19	0	67	3
	3	BALB/c Thy → (C57BL/6 × BALB/c)F_1 Nu	(BALB/c × C57BL/10)	1	39	2	10	13
				5	82	15	27	14
				10	88	20	38	9
	4	(BALB/c × C57BL/10)F_1	(BALB/c × C57BL/10)	1	59	0	15	5
				5	90	2	33	2
				10	87	6	45	7
2	5	B6 Thy → (C57BL/6 × BALB/c)F_1 Nu	(BALB/c × C57BL/10)	1	14	0	12	1
				5	36	0	32	0
				10	61	0	66	1
	6	(BALB/c × C57BL/10)F_1	(BALB/c × C57BL/10)	1	21	0	19	2
				5	57	0	37	6
				10	61	0	34	7

* (C57BL/6 × BALB/c)F_1 nude mice were grafted with thymus glands of the indicated parent. After immunization in vivo, spleen cells from these mice were stimulated in vitro with Sendai virus-treated F_1 stimulator cells as described (Materials and Methods).

‡ As in Table I.

individual variation has been observed by at least one other group using bone marrow chimeric mice (7).

These results are in agreement with recent results of Zinkernagel et al. (3, 4), with the notable exception that considerable individual variation in the degree of preferential lysis mediated by CTL from F_1 nude mice implanted with parental thymus glands was observed. It is clear that the thymus does not impose an absolute restriction on T cells because precursor T cells that can recognize virus in association with MHC antigens of the parental haplotype not represented in the thymus graft are present in parental Thy → F_1 nude mice. The results available from the three types of thymus-grafted nude mice examined to date, i.e., B Thy → A nude (Table I) (4), (A × B)F_1 Thy → A nude (4), and A Thy or B Thy → (A × B)F_1 nude (Table II) (3, 4) are more consistent with the concept that the MHC restriction of CTL is determined by the genotype of the T cells rather than the haplotype of the implanted thymus. In the case of the A Thy or B Thy → (A × B)F_1 nude mouse, the implanted thymus could select those T cells that were already committed to recognize antigen in association with the MHC antigens of the thymus. Those T cells that can recognize antigen in association with the other parental MHC antigens are presumably not expanded, or might be eliminated in the thymus. This selection hypothesis does not, however, provide an adequate explanation for the capacity of nude mice grafted with allogeneic thymus glands to mount a CTL response restricted for the host haplotype, nor does it readily explain the variability in responsiveness of F_1 nude mice implanted with parental thymus glands. The possibility also exists that other regulatory mechanisms, e.g., active suppression (8), are operative in some thymus-grafted nude mice which interfere with full maturation of T cells restricted to MHC antigens of the parental haplotype not represented on the grafted thymus. Furthermore, and in contrast to data from the chimera model (1, 9–11), no evidence exists in the thymus-grafted nude mouse model to suggest that stem cells that mature in an allogeneic (Table I) (4) or semiallogeneic thymus (4) have the ability to recognize antigen in association with MHC antigens not already expressed by the stem cells.

These data have been generated using one set of MHC haplotype combinations, i.e., H-2^d and H-2^b. Obviously, thymus-grafted nude mice of several different haplotype combinations must be examined to substantiate these observations. Furthermore, it is possible that the CTL responses observed in these allogeneic and semiallogeneic thymus gland-grafted nude mice could represent the response of an infrequent clone(s) expanded through immunization in vivo and subsequent expansion in vitro. Although the magnitudes of the responses do not support this view, quantitative estimates of the frequency of CTL precursors in these animals need to be obtained to resolve this issue. Despite these caveats, the pronounced difference in the pattern of MHC-restricted CTL responses between the chimera model and the thymus-grafted nude mouse model raises critical questions concerning the mechanism by which self MHC recognition by T cells is acquired and the role of the thymus in this process.

Summary

The in vitro secondary cytotoxic T lymphocyte (CTL) response to Sendai virus-treated stimulator cells by primed spleen cells from thymus gland-grafted nude mice was examined. BALB/c (H-2^d) nude mice grafted with allogeneic C57BL/10 (H-2^b) thymus glands developed CTL responses directed exclusively to Sendai virus-infected

H-2^d target cells. (C57BL/6 × BALB/c)F_1 nude mice grafted with thymus glands of either parent developed CTL responses preferentially against infected target cells expressing the MHC antigens present in the parental thymus graft, but also had detectable activity for infected target cells of the parental haplotype not expressed in the thymus. These results provide evidence against the concept that self recognition by MHC-restricted CTL is directed exclusively by the MHC type of the thymus.

Received for publication 11 September 1980 and in revised form 7 October 1980.

References

1. Zinkernagel, R. M. 1978. Thymus and lymphohemopoietic cells: their role in T cell maturation in selection of T cells H-2-restriction-specificity and in H-2 linked Ir gene control. *Immunol. Rev.* **42:**225.
2. Fink, P. J., and M. J. Bevan. 1978. H-2 antigens of the thymus determine lymphocyte specificity. *J. Exp. Med.* **148:**766.
3. Zinkernagel, R. M., A. Althage, and G. Callahan. 1979. Thymic reconstitution of nude F_1 mice with one or both parental thymus grafts. *J. Exp. Med.* **150:**693.
4. Zinkernagel, R. M., A. Althage, E. Waterfield, B. Kindred, R. M. Welsh, G. Callahan, and P. Pincetl. 1980. Restriction specificities, alloreactivity, and allotolerance expressed by T cells from nude mice reconstituted with H-2-compatible or -incompatible thymus grafts. *J. Exp. Med.* **151:**376.
5. Metcalf, D. 1966. The Thymus. Springer-Verlag New York, Inc., New York. 1.
6. Braciale, T. J. 1977. Immunologic recognition of influenza virus infected cells. I. Generation of a virus strain-specific and a cross reactive subpopulation of cytotoxic T cells in the response to type A influenza virus of different subtypes. *Cell. Immunol.* **33:**423.
7. Blanden, R. V., and M. E. Andrew. 1979. Primary anti-viral cytotoxic T-cell responses in semiallogeneic chimeras are not absolutely restricted to host H-2 type. *J. Exp. Med.* **149:** 535.
8. Smith, F. I., and J. F. A. P. Miller. 1980. Suppression of T cells specific for the nonthymic parental H-2 haplotype in thymus-grafted chimeras. *J. Exp. Med.* **151:**246.
9. Matzinger, P., and G. Mirkwood. 1978. In a fully H-2 incompatible chimera, T cells of donor origin can respond to minor histocompatibility antigens in association with either donor or host H-2 type. *J. Exp. Med.* **148:**84.
10. Sprent, J. 1978. Role of H-2 gene products in the function of T helper cells from normal and chimeric mice *in vivo*. *Immunol. Rev.* **42:**108.
11. Erb, P., P. Vogt, T. Matsunaga, A. Rosenthal, and M. Feldmann. 1980. Nature of T cell-macrophage interaction in helper cell induction *in vitro*. III. Responsiveness of T cell differentiating in irradiation or allophenic chimeras depends on the genotype of the host. *J. Immunol.* **124:**2656.