Brief Definitive Report

THYMIC RECONSTITUTION OF NUDE F_1 MICE WITH ONE OR BOTH PARENTAL THYMUS GRAFTS*

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Thymus-derived lymphocytes (T cells) have two outstanding characteristics that distinguish them from other lymphocytes: (*a*) they express two specificities, one for self-antigens, the major transplantation antigens (H) coded by the major histocompatibility gene complex (MHC), and a second specificity for foreign antigenic determinants. (*b*) T cells must undergo differentiation or maturation in the thymus (1, 2). Apparently, an important step in T-cell differentiation in the thymus is the selection of T-cells' restriction specificity for self-H. This interpretation stems from experiments with chimeras formed by lethally irradiating parental type mice and reconstituting them with F_1 stem cells: the maturing F_1 T cells expressed predominantly the restriction specificities for the recipient parental MHC type (3–8). Alternatively, adult F_1 mice that were thymectomized, lethally irradiated, reconstituted with bone marrow, and then engrafted with a parental thymus had T cells that were restricted predominantly to the thymus donors' H-2 (4–8).

The present study first extends these observations to nude mice that are born without a thymus and therefore do not develop functional T cells and second, attempts to study the possibility that suppression may be responsible for the apparent influence of the radioresistant portion of the thymus on T-cell restriction specificities.

We tested the immunocompetence and restriction specificities expressed by lymphocytes from F_1 nude mice reconstituted with both parental thymus grafts; our expectation was that suppression of the expression of T-cell restriction specificity should result either in complete immunoincompetence or emergence of only one of the two possible sets of restriction specificities. Nude F_1 mice that simultaneously received thymus grafts from both parents developed spleen cells restricted to both parental H-2 types. These results are compatible with the idea that the thymus' influence on T-cell restriction is via positive selection rather than by suppression.

Materials and Methods

Mice. BALB/c nu/nu female mice were purchased from the nude mice breeding facility of the University of California at San Diego thanks to the generosity of Dr. G. Sato and D. Mackensen. These mice have been described in references 9 and 10. The (C57BL/6 × BALB/c)F_1 nu/nu mice were derived from fifth backcross generation C57BL/6 nu/+ and seventh backcross generation BALB/c nu/nu; they were generously donated by Doctors J. Hedrick and J. Watson (11,12). None of the nude mice generated detectable virus-specific cytotoxic T cells.

* Publication No. 1755 from the Departments of Immunopathology and Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, Calif. Supported by U. S. Public Health Service grants A1-13775 and A1-00273.

To form thymus chimeras, 6- to 8-wk-old nude mice were transplanted with thymus grafts from 15- to 17-d-old fetuses under the kidney capsule.

Several protocols were tried. The only successful method for obtaining nude F_1 mice with functional grafts of both parents was to transplant 15- to 17-d-old fetal thymus lobes from one parental strain under the left kidney capsule and from the other parental strain under the right kidney capsule on the same day. When, instead, F_1 nude mice were transplanted first with either BALB/c or C57BL/6 fetal (17–19 d) thymus lobes and 1–4 wks later received the second parental grafts, only the first grafts were functional. Similarly, if one parental graft was older (e.g., <24 h after birth) than the other one (16- to 18-d-old fetal grafts), only the older graft was histologically intact and functional; the younger graft left a connective tissue scar in the recipients kidney. In all cases the functional test and the observed restriction specificity of mature T cells correlated with the engrafted and histologically normal thymus.

Virus, Immunization, ^{51}Cr-Release Assay, and H-2 Typing Procedures. Nude mice reconstituted with thymus grafts for 5–20 wk or control mice were infected with ≅5×10^6 plaque-forming units of vaccinia virus. Spleen cells from these mice were tested 6 d later for cytotoxic activity on vaccinia-infected or -uninfected target cells (4, 13). The established fibroblast or fibrosarcoma cell lines from C3H origin (L929, H-2^k), B10.D2 (D2, H-2^d), and C57BL (MC57G, H-2^b) have been described previously (4, 13). Test duration (6 or 16 h) and the percentage of spontaneous ^{51}Cr release are indicated in the table legends. Statistical comparisons used Student's *t* test. Each chimera was typed for H-2 as described previously (14).

Results and Discussion

T cells from F_1 nude mice reconstituted with thymus grafts from one parental strain are restricted in specificity to lyse only infected target cells sharing the H-2 type of the donors' thymus (Table I, exp. 2). If suppression was directed against T-cell receptors specific for self-H of the second parent in such thymically reconstituted mice, one might expect that F_1 nude mice that were reconstituted simultaneously with thymus grafts from both parental strains would become immunoincompetent (caused by reciprocal suppression of restriction specificities) or would express only one set of parental restriction specificities (the one that matures earliest or is dominant).

To test this proposition, (C57BL/6 × BALB/c) (H-2^b × H-2^d)F_1 nude mice were transplanted on the same day with thymus lobes taken from fetuses of both parental strains; C57BL/6 lobes were inserted under each recipient's left kidney capsule, and BALB/c lobes were placed under the right kidney capsule (Table I, exp. 1). Other (C57BL/6 × BALB/c)F_1 nude mice were grafted with fetal thymus lobes of either C57BL/6 or BALB/c origin (Table I, exp. 2). The transplant recipients were infected with vaccinia virus 8–10 wk later. 6 d after infection, virus-specific cytotoxic activity of the spleen cells was tested on appropriate target cells. Spleen cells from the transplanted mice with histologically normal grafts from both parents lysed infected target cells of both parental types (Table I, exp. 1). Infected *H-2^b* and infected *H-2^d* targets were lysed about equally well. Spleen cells from unmanipulated control mice (C57BL and BALB/c) were about three times more active than those from the reconstituted nude mice. Nude (C57BL/6 × BALB/c)F_1 mice that were reconstituted with thymus grafts of only one parent (Table I, exp. 2) generated virus-immune T cells that were restricted to the H-2 type of the thymus donor alone. This was invariably true as well for nude F_1 mice reconstituted with thymus grafts from both parents but retaining only one histologically functional thymus. As shown in Table I, exp. 2, the restriction specificity expressed by (C57BL/6 × BALB/c)F_1 nude mice reconstituted with BALB/c (*H-2^d*) thymus grafts was significant for infected *H-2^d* targets only. This activity was about one-half of that of the unmanipulated control

TABLE I

*Restriction Specificities of Virus-specific Cytotoxic T Cells from (C57BL/6 × BALB/c)F_1 NU/NU Mice Reconstituted with Fetal Thymus Grafts from One or Both Parental Strains**

Recipient nu/nu	Thymus donors	Ratio of spleen cells to target cells	Percentage of specific release from infected target cells‡ H-2^d (D2)		H-2^b (MC57G)	
Exp. 1						
(C57BL × BALB/c)F_1 (*H-2^b* × *H-2^d*)	{BALB/c (*H-2^d*) plus C57BL (*H-2^b*)}	40	[96]		[88]	
		13	[64]		[43]	
		4	[27]		[8]	
Controls (*a*) C57BL		40	13		[80]	
		13	11		[79]	
		4	5		[59]	
(*b*) BALB/c		40	[95]		0	
		13	[100]		0	
		4	[44]		0	
Exp. 2			Vacc.	Nor.	Vacc.	Nor.
(C57BL × BALB/c)F_1	BALB/c (*H-2^d*)	40	[63]	1	[8]	4
		13	[36]	3	3	2
		4	15	1	2	1
(C57BL × BALB/c)F_1	C57BL (*H-2^b*)	40	10	8	[58]	0
		13	7	5	[27]	0
		4	8	4	[12]	1
Controls (*a*) C57BL		40	11	10	[54]	0
		13	15	10	[28]	0
		4	0	5	[13]	0
(*b*) BALB/c		40	[52]	1	3	3
		13	[38]	4	1	1
		4	[18]	2	1	0

Vacc., vaccine; Nor., normal.

* Recipient (C57BL/6 × BALB/c)F_1 nu/nu mice were transplanted with 15- to 18-d fetal thymus lobes under the kidney capsule. 8–10 wk later the thymus chimeras were infected intravenously with ≅5 × 10^6 plaque-forming units of vaccinia virus. 6 d later the mice were killed; their spleen cells were tested for cytotoxic activity, and H-2 type (>95% of F_1 origin), and the thymus grafts were examined histologically.

‡ ^{51}Cr-release test conditions. Exp. 1: test duration 16 h; spontaneous release from infected D2:37%; from infected MC57G: 31%. Exp. 2: test duration 6 h; spontaneous release from infected D2:18% from infected MC57G:21%. Results that are significantly greater than those obtained by normal or histoincompatible immune spleen cells are boxed ($P < 0.05$).

mice and was at least 20–30 times greater on infected *H-2^d* targets than on infected *H-2^b* targets. The lytic activity of spleen cells from F_1 nu/nu mice with a BALB/c thymus on infected H-2^b targets was very low, although definitely greater than spleen cells from unmanipulated BALB/c (*H-2^d*) mice; but this small activity was of questionable significance because both infected and uninfected targets were lysed. In the symmetrical combination, in which C57BL/6 (*H-2^b*) thymus lobes reconstituted F_1 nudes, spleen cell activity was again >30 times greater on syngeneic infected *H-2^b* targets than on the infected thymus-incompatible *H-2^d* targets. In this case the ^{51}Cr release by control spleen cells from C57BL mice on infected *H-2^d* targets was similar to that by chimeric lymphocytes, and both chimeric and control mice lysed uninfected targets to similar extents.

The present results confirm, in thymus-reconstituted nude mice, our and Bevan's earlier experiments in irradiation bone marrow and indicate that suppression does not explain the apparent thymic selection of T cells' restriction specificity in chimeras. These results are also in agreement with the finding that nude F_1 mice reconstituted

with thymus grafts from the parent that responds to collagen will, whereas those reconstituted with thymuses from the nonresponder parent will not, respond to collagen with an IgG response (12).

Our data confirm the finding (3–6) that the restriction of primary virus-specific cytotoxic T cells from *H-2*^{d} - *H-2*^{b} chimeras is, if experiments are thoroughly controlled (15), of comparable specificity as that expressed by unmanipulated control mice. It is clear that all immunological specificity is relative; therefore restriction specificity of cytotoxic T cells cannot be absolute. In fact, it has been shown that lymphocytes from H-2-incompatible irradiation bone marrow chimeras may express cytotoxic activities restricted to the tolerated H-2, that was not expressed in the thymus, upon secondary or tertiary restimulation against minor histocompatibility antigens (16). These and comparably highly selective experiments involving negative filtration of lymphocytes through irradiated allogeneic recipients to eliminate alloreactivity and subsequent sensitization against antigens presented together with the same alloantigens (17, 18, see also 19) cannot be used to compare or quantitate relevant precursor cells. Therefore, results of this type do not distinguish between the two models of T-cell recognition: a single receptor, for a neoantigenic determinant formed between self-H and foreign antigens versus two receptor sites, one for self-H and one for foreign antigen. As shown previously in thymus or irradiation bone marrow chimeras (3–8), in negative selection experiments (18) and here, it is remarkable that the degree of restriction in primary antiviral responses by thymic chimeras is comparable to that by normal mice. Our results are from chimeras in which antigen presentation is optimal in association with both H-2 haplotypes involved in a given chimera; they reflect restricted T-cell activity generated during an acute primary antiviral response in vivo. In absence of a reliable assay to estimate the relative frequency of precursor cells in a defined in vitro system, the relative activity found in these chimeras, where no selection should occur at the level of sensitization, gives the best estimate of relative precursor frequencies we can obtain. T-cell activity, restricted to the thymic H-2, is at least 30- to 50-fold greater than for the second parental H-2 type that is not expressed in the thymus. This does not, however, exclude the possible presence of rare precursor T cells that may be restricted (by cross-reactivity?) to the MHC type absent from the thymus and may be boosted under selective conditions to become measurable (20). We therefore feel, to understand the general principles of T-cell restriction and recognition, it is more important to further analyze and understand the high frequency of T cells that are restricted to thymic MHC rather than to generalize from a rare exception.

Received for publication 26 March 1979.

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