SELF-RECOGNITION SPECIFICITY EXPRESSED BY T CELLS FROM NUDE MICE

Absence of Detectable Ia-restricted T Cells in Nude Mice That Do Exhibit Self-K/D-restricted T Cell Responses

By ADA M. KRUISBEEK, MARY L. DAVIS, LOUIS A. MATIS, and DAN L. LONGO

From the Medicine Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

The T cell receptor repertoire for self major histocompatibility complex (MHC)[1] antigens and the Ir gene phenotype of responding T cells are not genetically determined but are acquired during ontogeny and are dictated by the MHC gene products of the host in which the T cells mature (1–3). The host element that determines the T cell self-recognition repertoire is the subject of much controversy, particularly when one considers separately the two major subsets of T cells, the H-2 K/D region–specific cytotoxic T lymphocytes (CTL) and the I region–restricted proliferating and helper T cells. On the one hand, studies with radiation-induced bone marrow (BM) chimeras or thymus-engrafted nude mice have indicated that peripheral T cells, both K/D and I region specific, recognize conventional antigens (Ag) in association with thymic MHC gene products (4–11), supporting a role for the thymus in the development of the MHC restriction specificity of T cells. However, several other investigations have failed to confirm such a unique role for the thymus. First, in radiation-induced BM chimeras, peripheral CTL with self-specificity for both thymic and extrathymic H-2 K/D Ag were observed (12–15). In vitro generation of CTL restricted to extrathymic K/D determinants was dependent on the addition of an exogenous source of T cell help (interleukin 2 [IL-2]) (13) or antigen-presenting cells (APC) carrying thymic H-2 I region determinants (12). Second, in congenitally athymic nude mice engrafted with an allogeneic thymus, splenic CTL with self-recognition specificity for both thymic and extrathymic (i.e., nude host) H-2 K/D Ag were observed (16). Again, in vitro generation of peripheral CTL specific for extrathymic K/D Ag was dependent on the addition of IL-2, while the generation of CTL restricted to thymic K/D determinants was not (16). The CTL system used in these studies, a trinitrophenyl (TNP)-modified self response, was shown

Address correspondence to A. Kruisbeek, Medicine Branch, National Cancer Institute, National Institutes of Health, Building 10, Room 12N226, Bethesda, MD 20205.

[1] *Abbreviations used in this paper:* APC, antigen-presenting cell; BM, bone marrow; CFA, complete Freund's adjuvant; Con A SN, concanavalin A–induced spleen cell supernatant; CTL, cytotoxic T lymphocyte; cyto *c*, pigeon cytochrome *c*; FCS, fetal calf serum; Glϕ, poly(Glu^{55}Lys^{36}Phe^{9})n; IL-2, interleukin 2; LN, lymph node; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; PPD, purified protein derivative of *Mycobacterium tuberculosis*; TNP, trinitrophenyl.

J. Exp. Med. © The Rockefeller University Press · 0022-1007/84/09/0839/19 $1.00 839
Volume 160 September 1984 839–857

to be strictly dependent on activation of I region–restricted T helper cell precursors (12, 17). It was inferred from these findings that CTL precursors with self-specificity for extrathymic K/D determinants have developed in chimeras and nude mice but that the development of I region–restricted T cells is strictly thymic dependent.

The present study examines the MHC restriction of nude mouse T cell precursors specific for either K/D or I region determinants. Since the discovery of IL-2, several investigators reported the existence of an extrathymic CTL repertoire in nude mice (18–21), but no analysis of an I region–restricted repertoire in unmanipulated nude mice has been reported. To examine this issue we constructed chimeras of irradiated parental mice receiving a mixture of F_1 nude mouse (6–8 wk old) spleen and BM precursor cells. The donor inoculum was deliberately not treated with anti-Thy-1 plus complement (C), so that any MHC-committed precursor T cells were allowed to differentiate and expand in the normal parental recipient. 3 mo after reconstitution, the chimeras were immunized with several protein antigens in complete Freund's adjuvant (CFA) in the footpads and their purified draining lymph node (LN) T cells tested 10 d later for the ability to recognize Ag on APC of either parental haplotype. Also, their splenic and LN primary TNP-specific CTL responses were tested with TNP-modified stimulator cells of either parental haplotype. The results demonstrate that T cell proliferative responses of these F_1 (nude) → parent chimeras were restricted solely to recognizing parental host I region determinants as self and expressed the Ir gene phenotype of the host. CTL responses, on the other hand, were generated (in the presence of IL-2) with TNP-modified stimulator cells of either parental haplotype. Thus, this study suggests that in nude mice self-K/D-specific CTL precursors have indeed developed extrathymically, but self I region–restricted T cells are absent in nude mice. Therefore, development of I region restriction is strictly dependent on intrathymic differentiation.

Materials and Methods

Mice. C57BL/10Sn (B10), B10.D2, and B10.A mice were obtained from The Jackson Laboratory, Bar Harbor, ME. C3H/HeN (C3H), C57BL/6 (B6), (C3H × B6)F_1, (B10 × B10.D2)F_1, (C3H × B6)F_1 (nude), and (B10 Scn × B10.D2)F_1 (nude) mice were obtained from the Small Animal Section, Veterinary Resources Branch, Division of Research Services, NIH and used at 6–8 wk of age.

Construction of Chimeras. Homozygous recipients were given 950 rad from a ^{137}Cs source at 128 rad/min and were reconstituted within 6 h with 10^7 F_1 BM cells along with (in some experiments) 2×10^7 F_1 spleen cells administered intravenously. The F_1 cells were from donors of the following kind: (*a*) normal F_1 donors that had been depleted of T cells in vivo by antithymocyte globulin and cortisone treatment followed by an in vitro treatment with anti-Thy-1.2 antibody (Ab) plus C, as previously described (22); (*b*) F_1 nude heterozygotes that are phenotypically normal and not T cell depleted in vivo or in vitro; (*c*) F_1 nude mice, also untreated. Chimeras were used 3 mo after reconstitution and are designated as donor → irradiated recipient. To ensure that all spleen and LN cells were of donor origin, lymphoid cells from chimeras were typed by cytotoxicity and each test for function included a group in which the responding cells were treated with anti-K^kD^k plus C [for (C3H × B6)F_1 → B10 chimeras], anti-K^d plus C [for (B10 × B10.D2)F_1 → B10 chimeras], or antiK^bD^b plus C [for (B10.D2 × B10)F_1 → B10.D2 chimeras]. Such treatment always completely removed any T cell function, indicating that the responding cells were of donor F_1 origin. Monoclonal Ab used for this treatment were: 15-1-5P (anti-

K^kD^k, cross-reactive on $D^{d,s,q,r}$) (23); 15-5-5S (anti-D^k, cross-reactive with $K^{d,f}$) (23); and 28-8-6S (anti-K^bD^b) (24) and were obtained from American Type Culture Collection, Rockville, MD.

Antigens and Immunization. Purified protein derivative of *Mycobacterium tuberculosis* (PPD) (Connaught Medical Research Laboratory, Willowdale, Ontario) was used in culture at 20 μg/ml and pigeon cytochrome *c* (cyto *c*) (Sigma Chemical Co., St. Louis, MO), poly($Glu^{56}Lys^{35}Phe^9$)n (GLϕ) (Miles-Yeda, Rehovot, Israel), (T, G)–A—L (Miles-Yeda), and calf skin collagen (Sigma Chemical Co.) were used in culture at 100 μg/ml. All immunizations were carried out by injecting into each hind footpad 0.1 ml of an emulsion that contained a 1:1 mix of Ag in phosphate-buffered saline (1 mg/ml) and CFA (Difco Laboratories, Detroit, MI) containing *M. tuberculosis* strain H37 Ra.

T Cell Proliferation Assay. All treatments of cells were performed in Hanks' balanced salt solution (BSS) supplemented with 5% fetal calf serum (FCS), 10 mM Hepes and antibiotics. Responder T cells were isolated from draining LN, from Ag-primed chimeric (B10 × B10.D2)F_1 → parent mice by passage over nylon wool columns (25) followed by anti-I-A plus C treatment. Nylon-passed cells were adjusted to 10×10^6 cells/ml in medium containing 1 μg/ml of MK-D6 protein A–purified Ab (anti-I-A^d) (26) and incubated at 4°C for 45 min. After one wash, the cells were resuspended at 10×10^6 ml in a 1:10 dilution of rabbit C (Lo-Tox; Cedarlane Laboratories, Westbury, NY) and incubated for 35 min at 37°C. After two washes the cells were resuspended at 4×10^6/ml in complete tissue culture medium (see below). The effectiveness of this treatment was demonstrated in each experiment by the inability of such purified T cells to respond to soluble Ag in the absence of APC (see Results).

Where indicated, LN preparations not treated with anti-Ia plus C were used as the responding population along with soluble Ag. When LN T cells were used, spleen cells irradiated with 2,000 rad were used as a source of APC. Spleen cells were either added along with soluble Ag or after Ag pulsing. 10^7 spleen cells/ml were exposed to 100 μg/ml Ag for 1 h at 37°C and Ag not associated with cells was removed by five washes in Ag-free medium. Cultures consisted of either 4×10^5 LN cells plus soluble Ag; 4×10^5 LN T cells plus 2×10^5 nonpulsed spleen cells plus soluble Ag; or 4×10^5 LN T cells plus 2×10^5 Ag-pulsed spleen cells, all in a final volume of 0.2 ml complete tissue culture medium consisting of half RPMI, half Eagle's Hanks' amino acid (27) medium supplemented with 10% FCS (lot 100402; Hy Clone Tissue Culture Products, Sterile Systems, Logan, UT), 2 mM glutamine, pencillin (100 U/ml), streptomycin (100 μg/ml), 2-mercaptoethanol (5×10^{-5} M), and Na pyruvate (0.11 mg/ml). Triplicate cultures were set up in flat-bottomed microtiter plates. On day 3, 1 μCi of [^{3}H]thymidine (6.7 Ci/mM) (New England Nuclear, Boston, MA) was added per well and the cultures were harvested 16–18 h later. [^{3}H]thymidine incorporation was measured in a liquid scintillation counter and data are expressed as the arithmetic mean counts per minute ± standard error (SE) of the mean.

In Vitro Generation of TNP-specific CTL Responses. Mixed lymphocyte cultures of 4×10^6 splenic or LN responder cells and 2×10^6 2,000 rad–irradiated, TNP-modified splenic stimulator cells were performed in 2 ml of complete tissue culture medium (see above) in 24-well tissue culture plates (Costar, Data Packaging, Cambridge, MA). TNP modification was performed with 10 mM trinitrobenzene sulfonate as described (28). The ^{51}Cr-release assay was performed on day 5 or 6 with TNP-modified, ^{51}Cr-labeled, Con A–induced splenic blasts as target cells. The percent specific ^{51}Cr release = 100 × [(experimental − spontaneous release)/(detergent − spontaneous release)]. Data shown are the means of triplicate determinations using 5×10^3 target cells (SD < 8%) and are representative of at least five separate experiments. Specific ^{51}Cr release from unmodified targets was always <4%. Maximum ^{51}Cr release values in detergent ranged from 4,000 to 9,000 cpm and spontaneous release values were always <25%.

In some experiments, concanavalin A supernatant (Con A SN) from rat spleen cell cultures was used as a source of IL-2. Con A SN (rat T cell Polyclone; Collaborative Research, Inc., Lexington, MA) was always supplemented with 0.1 M α-methyl-D-man-

noside to prevent mitogenic effects of the remaining Con A and was used at a 10% (vol/vol) concentration.

Results

Comparison of the I Region Restriction Specificity and Ir Gene Phenotype of F_1(Nude) → Parent and F_1(Nude/+) → Parent Radiation Chimeras. To examine athymic nude mice for the presence of an I region–restricted repertoire, we made radiation chimeras of lethally irradiated parental mice reconstituted with either BM alone or a mixture of BM and spleen cells from F_1 nude mice or from their F_1 heterozyogous (nude/+) normal litter mates. Thus, if any I region–restricted precursor T cells existed in the donor inoculum, they might expand and differentiate in a normal environment with a functioning thymus. The chimeras were immunized with a variety of soluble Ag in CFA in the hind footpads at 3 mo after reconstitution, and the proliferative response of lymph node (LN) T cells to these Ag was tested 8–10 d later. The prototypical Ir gene phenotypes of the mouse strains used in this study are shown in Table I.

To document that BM (with or without spleen cells) from nude mice is fully capable of reconstituting T cell immunity in lethally irradiated mice, we compared T cell proliferative responses of irradiated B6 recipients of F_1 nude mouse cells with those of recipients of T cell–depleted normal F_1(nude/+) cells. As shown in Table II, a PPD response occurred in (C3H × B6) F_1 → B6 chimeras, regardless of whether the donor inoculum consisted of F_1 nude BM or T cell–depleted F_1(nude/+) BM. Also, the addition to the donor inoculum of spleen cells of the same donor as the BM (i.e., nude spleen plus nude BM; T cell–depleted nude/+ spleen plus T cell–depleted nude/+ BM) did result in equally good reconstitution. Pretreatment of the responding LN T cells before assay with anti-H-2K^k plus C completely abolished the subsequent proliferative response (data not shown), demonstrating that the functional T cells were of donor origin. In summary, these experiments establish that nude mouse BM (with or without spleen cells) is as competent as T cell–depleted normal mouse BM in reconstituting T cell responses in lethally irradiated mice.

Interestingly, these chimeras displayed the Ir gene phenotype of the host: Regardless of the H-2 type of the donor inoculum, a good response to (T,G)–

TABLE I

H-2 and Ir Gene Phenotype of the Haplotypes Used in this Study

Strain	H-2	Antigens				
		PPD	TGAL	GLϕ	Collagen	Pigeon cyto *c*
B10, B6	b	+	+	−	+	−
C3H	k	+	−	−	−	+
B10.D2	d	+	+	+	−	−
B10.A	a	+	−	−	−	+
(B6 × C3H)F_1	(b × k)	+	+	+	+	+
(B10 × B10.D2)F_1	(b × d)	+	+	+	+	−

TABLE II

Comparison of the T Cell Proliferative Responses in $F_1 \rightarrow$ Parent Chimeras Receiving Either F_1(Nude) or T Cell–depleted F_1(Nude/+) Donor Cells

LN Cells*		Antigens‡				
Donor	→Recipient	None	PPD	TGAL	GLϕ	Pigeon cyto *c*
(B6 × C3H)F_1 (nude) (BM)	→B6	1,277 ± 170	48,270 ± 3,790	81,355 ± 4,953	1,845 ± 446	1,566 ± 170
(B6 × C3H)F_1 (nude) (BM plus spleen)	→B6	3,038 ± 1,323	83,520 ± 1,784	150,024 ± 6,440	2,256 ± 867	1,992 ± 444
(B6 × C3H)F_1 (nude/+) (T-depleted BM)	→B6	1,298 ± 718	67,368 ± 5,092	59,772 ± 7,361	1,358 ± 236	1,509 ± 587
(B6 × C3H)F_1 (nude/+) (T-depleted BM plus spleen)	→B6	3,434 ± 864	102,452 ± 4,066	152,434 ± 8,211	1,658 ± 394	1,796 ± 282
(B6 × C3H)F_1 (nude/+) (Control)		2,408 ± 219	101,373 ± 12,589	103,472 ± 6,731	69,731 ± 7,127	31,375 ± 2,818

A—L (to which the B6 host is a responder; see Table I) but no response to GLϕ and pigeon cyto *c* (to both of which the host is a nonresponder) was observed (Table II). With normal F_1 BM rigorously depleted of contaminating T cells, this result is to be expected (22). However, in $F_1 \rightarrow$ parent chimeras made with F_1 BM containing residual T cells, responses to Ag to which both the donor and the host are responders are developed (22 and below). These data suggest therefore that neither nude mouse spleen nor BM contains such T cells capable of generating known I region responses. To examine this issue more closely, we compared the T cell proliferative responses of (B10 × B10.D2)$F_1 \rightarrow$ B10 and (B10 × B10.D2)$F_1 \rightarrow$ B10.D2 chimeras, using either nude mice or nude/+ normal litter mates as donors. The donor inoculum from the nude/+ F_1 mice was in these experiments used without any preceeding T cell depletion procedure. In Table III it can be seen that in chimeras made with F_1(nude/+) BM or BM plus spleen, no host restriction was observed: in $F_1 \rightarrow$ B10 chimeras, responses to GLϕ were observed despite the fact that the host is a nonresponder; in $F_1 \rightarrow$ B10.D2 chimeras, collagen responses were observed, despite the nonresponder phenotype of the host. In contrast, the chimeras made with F_1(nude) BM or BM plus spleen strictly displayed the host Ir gene phenotype: positive collagen and negative GLϕ responses in F_1(nude) → B10 chimeras and positive GLϕ and negative collagen responses in F_1(nude) → B10.D2 chimeras. All chimeras generated full responses to PPD, an Ag to which both parental haplotypes are responders. Treatment of the $F_1 \rightarrow$ B10 chimeric T cells with anti-H-$2K^d$ plus C and of $F_1 \rightarrow$ B10.D2 chimeric T cells with anti-H-$2K^bD^b$ plus C abolished the proliferative response, establishing that the response was due to donor-derived T cells rather than residual host-derived T cells (data not shown).

The above experiments demonstrate that the I region–committed T cells present in F_1(nude/+) BM or BM plus spleen can expand in an irradiated host. However, these experiments do not address whether I region–committed splenic T cells alone can be expanded under the same circumstances and, therefore, do not reveal whether, if any I region–committed T cells had been present in the

TABLE III

Proliferating T Cells From F_1(Nude) → Parent Chimeras, But Not from Untreated F_1(Nude/+) → Parent Chimeras, Display the Ir Gene Phenotype of the Host

LN cells*		Antigens‡			
(B10 × B10.D2) donor untreated	→ Recipient	None	GLϕ	Collagen	PPD
(Nude/+) BM	→ B10	1,209 ± 271	88,693 ± 9,102	57,856 ± 4,340	137,819 ± 11,220
(Nude/+) BM plus spleen	→ B10	1,617 ± 311	73,368 ± 8,189	43,771 ± 2,056	118,252 ± 9,993
(Nude/+) BM	→ B10.D2	2,529 ± 629	79,955 ± 6,919	30,330 ± 2,941	104,383 ± 11,519
Nude/+) BM plus spleen	→ B10.D2	1,961 ± 215	96,424 ± 3,212	39,789 ± 3,212	126,511 ± 12,959
(Nude) BM	→ B10	3,126 ± 455	3,044 ± 511	28,951 ± 1,951	92,886 ± 8,178
(Nude) BM plus spleen)	→ B10	1,389 ± 550	1,456 ± 315	40,079 ± 2,829	110,629 ± 12,689
(Nude) BM	→ B10.D2	2,888 ± 550	67,488 ± 2,933	3,008 ± 268	155,588 ± 13,770
(Nude) BM plus spleen	→ B10.D2	2,014 ± 367	90,521 ± 11,204	2,621 ± 119	140,020 ± 9,191

* See footnote * to Table II.
‡ See footnote ‡ to Table II.
ǂ In contrast to Table II, the F_1(nude/+) donor inoculum had not been subjected to any T cell depletion procedures.

TABLE IV

Proliferating T Cells From F_1(Nude BM plus Nude Spleen) → Parent Chimeras But Not From F_1(Nude BM plus Nude/+ Spleen) → Parent Chimeras Display the Ir Gene Phenotype of the Host

LN cells*		Antigens‡				
Donor	→ Recipient	None	PPD	TGAL	GLϕ	Pigeon cyto *c*
(B6 × C3H)F_1 (nude BM)	→ B10.A	1,188 ± 211	73,858 ± 6,470	1,156 ± 195	1,229 ± 301	17,256 ± 1,410
(B6 × C3H)F_1 (nude BM plus nude spleen)	→ B10.A	1,855 ± 318	59,817 ± 4,237	1,988 ± 374	2,021 ± 258	11,882 ± 1,512
(B6 × C3H)F_1 (nude BM plus nude/+ spleen)	→ B10.A	2,036 ± 406	102,119 ± 11,314	81,129 ± 7,129	56,774 ± 6,312	23,314 ± 3,489

* See footnote * to Table II.
‡ See footnote ‡ to Table II.

F_1 nude spleen, these could have been expanded to detectable levels. To address this issue, an experiment was performed in which chimeras were made with F_1 nude BM (shown above to contain no I region–committed T cells; see Tables II, III) supplemented with either F_1 nude spleen or F_1(nude/+) spleen. As shown in Table IV, the addition of F_1 nude spleen to F_1 nude BM did not affect the results: the F_1 → B10.A chimeras displayed the Ir gene phenotype of the host, i.e., a good response to pigeon cyto *c* and no response to GLϕ and (T,G)–A—L, in both the presence or absence of nude spleen cells. However, when F_1(nude/+) spleen cells were added to the F_1(nude) BM, the chimeras displayed the Ir gene phenotype of the F_1 donor, i.e., responsiveness to GLϕ, (T,G)–A—L, and pigeon cyto *c*. Thus, these experiments document that I region–committed F_1(nude/+) T cells from spleen (or lymph node, data not shown) can indeed expand to easily detectable levels in an irradiated parental host also reconstituted with F_1(nude) BM. We therefore conclude that the inability of F_1(nude) BM plus

TABLE V

PPD-specific T Cells from F_1(Nude) → Parent Chimeras, But Not from Untreated F_1(Nude/+) → Parent Chimeras, Are Restricted to Self-recognizing I-A Ag of the Host

LN T cells*		Monoclonal Ab specificity‡	Antigen-presenting cells§			
(B10 × B10.D2) donor untreated BM plus spleen	→ Recipient		B10	PPD-pulsed B10	B10.D2	PPD-pulsed B10.D2
Nude/+	→ B10	None	1,047 ± 189	76,439 ± 6,045	1,314 ± 96	80,392 ± 5,818
		I-A^b		21,020 ± 1,919		85,546 ± 6,298
		I-A^d		77,488 ± 2,933		18,715 ± 333
Nude/+	→ B10.D2	None	2,211 ± 794	88,968 ± 10,172	1,813 ± 125	77,461 ± 8,257
		I-A^b		17,251 ± 2,611		68,633 ± 4,288
		I-A^d		92,581 ± 7,884		15,921 ± 932
Nude	→ B10	None	1,356 ± 99	56,699 ± 6,376	1,256 ± 137	2,086 ± 321
		I-A^b		8,286 ± 324		1,064 ± 117
		I-A^d		50,886 ± 1,297		1,468 ± 401
Nude	→ B10.D2	None	327 ± 10	3,012 ± 418	1,138 ± 401	65,980 ± 1,144
		I-A^b		1,758 ± 186		63,465 ± 6,010
		I-A^d		1,810 ± 71		14,348 ± 491

* T cells were isolated from LN from Ag-primed chimeras by nylon wool passage and anti-I-A plus C treatment and cultured at 2×10^5/0.2 ml culture for 4 d. The donor cells had not been subjected to any T cell depletion procedure.

‡ Protein A–purified Ab were used at 5 μg/ml, added at beginning of the culture period. Anti-I-A^b: 25-9-3S (24); anti-I-A^d: MK-D6 (26).

§ Spleen cells from either B10 or B10.D2 were added either pulsed or nonpulsed after 2,000 rad irradiation at 2×10^5 per culture. Without APC, the response of purified LN T cells to soluble PPD was <2,100 cpm in each experimental group.

spleen → parent chimeras to display the donor Ir gene phenotype for I region–restricted responses reflects the absence of I region–committed T cells in both the BM as well as the spleen of nude mice.

We next investigated whether F_1(nude) → parent chimeras were H-2 restricted in their response to PPD to which either parental haplotype is a responder. As shown in Table III, soluble PPD elicited a response in both F_1(nude) → B10 and F_1(nude) → B10.D2 chimeras. However, when donor F_1 APC were eliminated by treatment of the LN T cells with anti-I-A plus C and chimeric T cells tested for their ability to respond to PPD-pulsed spleen cells of either parental haplotype, a difference emerged: F_1(nude) → B10 T cells only responded to PPD-pulsed B10 spleen and F_1(nude) → B10.D2 T cells only responded to PPD-pulsed B10.D2 spleen (Table V). Thus, the genotypic F_1(nude) T cells had become restricted to recognition of host MHC determinants. Not surprisingly, the F_1(nude/+) → parent chimeras failed to demonstrate such host restriction: T cells from either F_1(nude/+) → B10 or F_1(nude/+) → B10.D2 chimeras responded well to PPD on spleen cells of either parental haplotype (Table V). Blocking of responses to PPD on B10 spleen with monoclonal anti-I-A^b and on B10.D2 spleen with anti-I-A^d (Table V) demonstrated that these responses were I region restricted.

Finally, the H-2 restriction of the Ir gene–controlled responses to GLϕ and collagen were tested. T cells isolated from F_1 → B10 and F_1 → B10.D2 chimeras were tested for their ability to respond to GLϕ-pulsed or collagen-pulsed spleen cells from either parental haplotype. B10 recipients of F_1(nude) BM plus spleen only responded to collagen-pulsed responder B10 spleen and not to GLϕ-pulsed

TABLE VI

T Cells From F_1(Nude) → Parent Chimeras Display the Ir Gene Phenotype of the Host and Recognize Antigens Under Ir Gene Control Only in Association with Host MHC Determinants

LN T cells*		Strain of APC‡	Antigen-presenting cells§		
(B10 × B10.D2) donor untreated BM plus spleen	→ Recipient		Nonpulsed	GLϕ pulsed	Collagen pulsed
Nude/+	→ B10	B10	888 ± 12	925 ± 329	23,983 ± 1,210
		B10.D2	1,299 ± 47	66,654 ± 1,231	654 ± 123
Nude/+	→ B10.D2	B10	919 ± 386	3,012 ± 418	25,980 ± 1,144
		B10.D2	1,138 ± 401	43,465 ± 840	810 ± 71
Nude	→ B10	B10	1,477 ± 38	1,767 ± 659	28,989 ± 4,071
		B10.D2	898 ± 21	1,813 ± 125	1,030 ± 304
Nude	→ B10.D2	B10	1,126 ± 103	971 ± 78	1,277 ± 49
		B10.D2	1,208 ± 48	41,351 ± 2,809	1,488 ± 301

* See footnote * to Table IV.

‡ B10 (nonresponder to GLϕ, responder to collagen) or B10.D2 (nonresponder to collagen, responder to GLϕ) spleen cells were used either nonpulsed or pulsed as indicated. Without APC, the response of purified LN T cells to soluble Ag was <2,400 cpm (for GLϕ responses) and <1,500 cpm (for collagen responses).

§ See footnote § to Table IV.

spleen of either parental haplotype (Table VI). In the reciprocal F_1(nude) → B10.D2 combination, only reponses to GLϕ-pulsed responder B10.D2 spleen were observed. In contrast, F_1(nude/+) → B10 chimeras and F_1(nude/+) → B10.D2 chimeras both responded equally well to GLϕ-pulsed B10.D2 and collagen-pulsed B10 spleen (Table VI), indicating that I region–committed F_1 T cells are not affected (negatively selected, suppressed, or restricted) by developing in the parental host environment.

In conclusion, chimeric recipients of F_1(nude) BM-plus-spleen generated T cells with I region specificity and Ir gene phenotype of the irradiated host, while recipients of F_1(nude/+) BM plus spleen (when not T cell depleted) generated T cells indistinguishable from the F_1 donor in both I region specificity and Ir gene phenotype. This suggests that nude spleen and BM do not contain any Ag-specific, proliferating I region–restricted T cells capable of being expanded in an irradiated host, an environment that is perfectly capable of allowing the expansion of I region–committed T cells from normal nude/+ donors.

Comparison of the TNP–Self CTL Repertoire in F_1(Nude) → Parent and F_1(Nude/+) → Parent Chimeras. The results presented thus far demonstrate the absence in nude mouse BM and spleen of T cells committed to self I region recognition, as evidenced by the failure of F_1(nude) → parent chimeras to display non-host I region–restricted recognition and non-host Ir gene phenotype. Several studies (16, 19) have described the existence of an extrathymic K/D region–restricted CTL repertoire in nude mice. Therefore, we proceeded to examine whether the same animals used for studying the existence of extrathymic I region–restricted cells, (i.e., F_1(nude) (BM plus spleen) → parent chimeras) could reveal the existence of extrathymic K/D region–restricted T cells. Primary in vitro TNP-self responses of spleen and LN cells from F_1(nude) → parent chimeras to TNP-modified stimulator cells of either parental haplotype were tested to address this issue.

In Fig. 1 it can be seen that splenic CTL from F_1(nude) → parent chimeras (same mice as used in Table III) are restricted to recognizing TNP in association

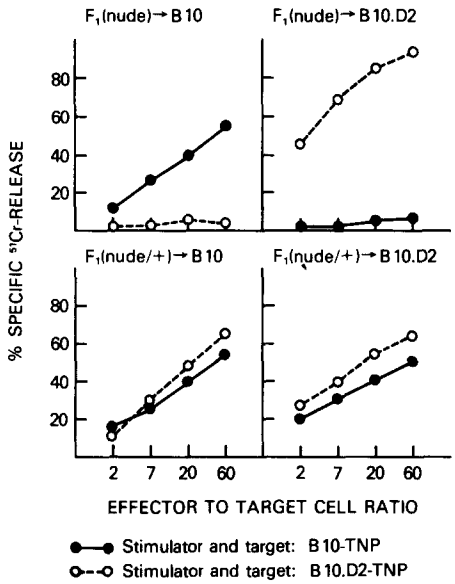

FIGURE 1. Comparison of the TNP-specific CTL responses by spleen cells from F_1(nude) → parent and F_1(nude/+) → parent chimeras. Spleen cells from chimeras made with a mixture of 2×10^7 spleen cells and 1×10^7 BM cells from either nude (B10 × B10.D2) donors or nude/+ (B10 × B10.D2) donors were tested for their ability to generate CTL responses to B10.TNP and B10.D2-TNP stimulator cells. The donor cells were not subjected to any T cell depletion procedure. Specific ^{51}Cr-release values represent the means of triplicate determinations (SD < 8%) and are representative of at least five separate experiments in which individual mice of each kind were tested. Absolute ^{51}Cr-release values were: 6,812 ± 204 cpm (detergent release B10-TNP); 1,231 ± 60 cpm (medium release B10-TNP); 8,442 ± 332 cpm (detergent release B10.D2-TNP); 1,769 ± 136 cpm (medium release B10.D2-TNP). ^{51}Cr-release values from unmodified B10 or B10.D2 target cells were <4% and are not included in the figure for simplicity.

with host MHC determinants: F_1(nude) → B10 chimeras only responded to TNP-modified B10 stimulators, while F_1(nude) → B10.D2 chimeras were only stimulated by TNP-modified B10.D2 stimulators. Identical results were obtained with LN cell CTL responses (data not shown). In contrast, F_1 → parent chimeras made with control F_1(nude/+) BM-plus-spleen (not T cell depleted) responded to TNP-modified stimulator cells of either parental haplotype. At face value, these data could be interpreted as indicating that F_1 nude mice lack self K/D-committed CTL precursors that can be expanded in the irradiated host and only provide noncommitted precursor cells that acquire self-recognition specificity for the MHC phenotype of the irradiated host similar to what was found for I region–restricted responses. In contrast, chimeras made with untreated F_1(nude/+) BM plus spleen contain donor-derived CTL precursors committed to recognizing F_1 MHC determinants that apparently have been expanded in the irradiated host. However, for TNP-specific CTL responses to occur, activation of I region–specific helper T cells is required (12, 17). Therefore, it is conceivable that the apparent host restriction of F_1(nude) → parent chimeras CTL responses is in fact imposed by the need for activation of host MHC–restricted, I region–specific T cells and the lack of non-host I region–restricted T cells (see above).

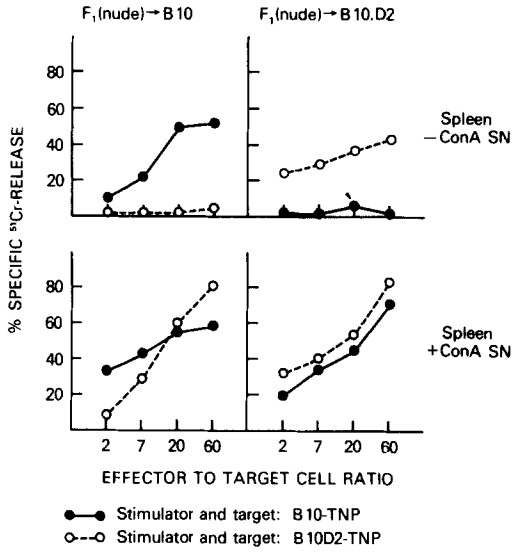

FIGURE 2. Comparison of the TNP-specific CTL responses by spleen cells from F_1 (nude) → parent chimeras in the presence and absence of exogenous helper factors. Spleen cells from chimeras made with a mixture of 2×10^7 spleen cells and 1×10^7 BM cells from nude (B10 × B10.D2)F_1 donors were tested for their ability to generate CTL responses to B10-TNP and B10.D2-TNP stimulator cells in the absence or presence of 10% (vol/vol) rat spleen cell Con A SN (supplemented with 0.1 M α-methyl-D-mannoside). Specific ^{51}Cr-release values represent the means of triplicate determinations (SD < 7%) and are representative of six separate experiments with individual mice. Absolute ^{51}Cr-release values were: 4,430 ± 201 cpm (detergent release B10-TNP); 748 ± 46 cpm (medium release B10-TNP); 4,354 ± 279 cpm (detergent release B10.D2-TNP); 1,015 ± 48 cpm (medium release B10.D2-TNP). ^{51}Cr-release values from unmodified B10 or B10.D2 target cells were <3% and not influenced by the addition of Con A SN.

Such dependency on the activation of I region–specific T cells in the TNP-self CTL response can be bypassed by adding a source of exogenous helper factors, such as Con A SN (12, 17). Upon reexamination of the CTL response of F_1-(nude) → parent chimeras in the presence of Con A SN, spleen cells were stimulated by TNP-modified stimulator cells of the non-host haplotype as well as the host haplotype (Fig. 2). Thus, when the need for I region activation is bypassed, F_1(nude) → parent chimeras reveal the presence of T cells with K/D specificity for both parental haplotypes.

To fully evaluate this apparent existence of extrathymic CTL precursors in F_1(nude) mice, it is important to determine whether the non-host-restricted CTL from F_1(nude) → parent chimeras are MHC restricted. Therefore, the H-2 specificity of the CTL generated in the presence and absence of Con A SN from F_1(nude) → parent chimeras was compared (Table VII). As was found above, in the absence of Con A SN, only CTL with self-recognition specificity for the host haplotype were generated. These CTL preferentially lysed the specific TNP-modified target and are therefore predominantly H-2 restricted. TNP-specific cross-reactive lysis is usually observed with spleen cells from normal and chimeric mice (29, 30) and increase as the magnitude of the specific lysis increases. In the presence of Con A SN, CTL were generated from the spleens

TABLE VII

Comparison of the H-2 Specificity of TNP-CTL Responses from (B10 × B10.D2)F_1(Nude) → Parent Chimeras in the Absence and Presence of Con A SN

Chimeric spleen cells: (B10 × B10.D2) donor untreated BM Plus spleen → Recipient	Con A SN	Effector to target cell ratio	Stimulator cells: / target cells:	B10-TNP* B10-TNP	B10-TNP* B10.D2-TNP	B10.D2-TNP* B10-TNP	B10.D2-TNP* B10.D2-TNP
Nude → B10	−	20		45[a]	13	0	−2
		7		34	9	−1	0
		2		19	0	−3	2
Nude → B10	+	20		69	21	18	75
		7		68	15	12	47
		2		36	6	7	29
Nude → B10.D2	−	20		7	5	12	59
		7		2	0	10	42
		2		0	0	3	29
Nude → B10.D2	+	20		54	15	20	82
		7		47	12	19	68
		2		31	5	10	44

* Values represent the percent of specific ^{51}Cr release of the indicated target cells (means of triplicate determinations; SD < 5%). The percent of specific ^{51}Cr release of target cells not modified with TNP was <4% in all groups.

of F_1(nude) → parent chimeras with self-recognition specificity for either parental haplotype (Table VII). The degree of cross-reactivity expressed by these non-host-restricted CTL is indistinguishable from that expressed by host-restricted CTL. Thus, the H-2 specificity of both types of CTL is comparable and it can be concluded that both the CTL with self-recognition specificity for the host MHC as well as the CTL with self-recognition specificity for the non-host MHC were H-2 restricted.

In conclusion, F_1(nude) → parent chimeras exhibit TNP-CTL responses with self H-2 specificity for both parental haplotypes. Given the previously reported existence of CTL precursors in nude mice (16, 18–21) these results most likely reflect the presence of CTL committed to self-K/D region recognition in nude mice and their subsequent expansion in the irradiated host. At the same time, the dependency of expression of this extrathymic CTL repertoire on exogenous helper factors, and the failure of F_1(nude) → parent chimeras to express non-host I region recognition, indicate that nude mice lack T cells committed to self I region recognition.

Discussion

The present study demonstrates an important difference between the pathways for generating K/D region–restricted CTL and I region–restricted proliferating T cells in F_1(nude) → parent chimeras. The F_1 CTL in the spleen and LN of F_1(nude) (BM plus spleen) → parent chimeras are capable of responding to TNP in association with H-2 determinants of either parental haplotype, provided the inherent need for I region activation is this response is bypassed by supplying an exogenous source of IL-2. In contrast, in the LN of the same F_1(nude) → parent chimeras, proliferating I region–restricted T cells are restricted solely to the recognition of various soluble antigens in association with host I region deter-

minants and express the host Ir gene phenotype. Since F_1(nude/+) (untreated) → parent chimeras did not display host I region restriction, these irradiated parental hosts are apparently fully capable of allowing expansion of I region–committed T cells present in the donor inoculum. Thus, it can be concluded that F_1(nude) donor cells used for constructing F_1(nude) → parent chimeras must lack I region–committed T cells. K/D region–committed CTL, on the other hand, could be easily detected in the present and other (16, 19) experiments using nude mice. Consequently, these results suggest that nude mice do have an extrathymic CTL repertoire but do not have an extrathymic I region–specific T cell repertoire, and raise the issue as to how the I region– and K/D region–specific T cell repertoire are differentially influenced by the host environment.

The finding of host restriction and host Ir gene phenotype of the I region–specific T cell repertoire of F_1 (nude) BM plus spleen → parent chimeras could be explained in at least two ways. Either the F_1(nude) donor inoculum contained no I region–committed T cells, or it contained quantities too low to be subsequently sufficiently expanded in the irradiated host. The latter possibility seems unlikely because even as late as 11 mo after reconstitution, we failed to detect any T cells restricted to donor I region determinants (data not shown). Also, even the few I region–committed T cells in an incompletely T cell–depleted normal F_1 BM donor inoculum succeed in expanding into easily detectable numbers in an irradiated parental host (22), i.e., in such chimeras, no host restriction is observed. Thus, our results more likely truly reflect the absence of any I region–committed T cells. At least superficially, this data is difficult to reconcile with several recent reports on IL-2 production by nude T cells (31–33), a response generally considered associated with activation of I region–restricted T cells. However, in these studies, either Con A (31, 32) or fully allogeneic and M1s-disparate stimulator cells (33) were used for induction of IL-2 production, so the IL-2 produced may not reflect activation of I region–specific T cells and, certainly, I region dependence of IL-2 production was not demonstrated. It should be noted also that the activation of K/D region–specific T cells can lead to IL-2 production (34). Another difference is that in our studies, the F_1(nude) donors were at most 6–8 wk old, whereas the age at which the frequency of IL-2-producing T cells in nude mice becomes significant is at least 4 mo (33). Experiments with F_1 → parent chimeras in which the F_1(nude) donors are at least 6 mo old are underway to test the contribution of aging on the appearance of I region–restricted cells in nude mice. In conclusion, until IL-2 production in responses dependent solely on I region activation is measured, the present results are most consistent with the notion that no I region–committed T cells exist in young nude mice, and consequently, that the differentiation of the I region–specific repertoire is a truly intrathymic event. The nude mouse does, however, have normal noncommitted precursors of I region–specific T cells, whose restriction specificity can be influenced by either an irradiated host in F_1(nude) → parent chimeras (present experiments) or by an allogeneic thymus graft (9, 11). F_1(nude) → parent chimeras are indistinguishable from F_1(nude/+) (T depleted) → parent chimeras in their host I region restriction and host Ir gene phenotype, and nude mice with an allogeneic thymus graft exhibit I region–specific T cell responses restricted to the thymic haplotype (9, 11). In conclusion, although

nude mouse BM provides early noncommitted precursor cells that can, in the appropriate environment, develop into I region–specific T cells, the young nude mouse environment does not allow for "education" of I region–specific T cells.

The capacity of F_1(nude) → parent chimeras to develop CTL restricted to recognizing antigen in association with both parental haplotypes is somewhat more complicated to explain. First, it is known that F_1 → parent chimeras made with T cell–depleted normal F_1 BM display both a host-restricted and (in the presence of exogenously added T cell help) a non-host-restricted CTL repertoire (13). Thus, one could explain the results with both F_1(nude) → parent and normal F_1 → parent chimeras as reflecting the "education" of non-host-restricted CTL on extrathymic, donor BM–derived elements in the chimeras (12, 13). A second explanation arises, however, when one takes into account the observations that in unmanipulated (19) and thymic-engrafted (16) nude mice, self-K/D–restricted CTL exist, as well as CTL alloreactive to the thymic H-2 haplotype in thymus-grafted nude mice (16). Consequently, one could argue that the non-host-restricted CTL observed in F_1(nude) → parent chimeras are the descendants of the self-H-2–committed CTL precursors present in the F_1(nude) donor inoculum that have matured into full competence in the irradiated parental host under the influence of the host's thymic factors but are not influenced by its H-2 haplotype. Both possibilities are compatible with the data presented here. Whichever explanation is correct, one has to conclude that the specificity of the K/D-restricted CTL repertoire can indeed be dictated extrathymically, either in the F_1(nude) → parent chimeric host or before that in the F_1(nude) donor. We favor the latter notion because it is fully compatible with multiple observations of K/D-specific CTL in nude mice (16, 18–21). As we (16) and several others (20, 32) have consistently failed to observe any CTL function in unmanipulated young nude mice, it follows that the differentiative pathway for extrathymic commitment to a certain H-2 specificity occurs before subsequent maturation and expansion under the influence of the thymus as provided by either a graft (16) or by the irradiated host. Experiments with thymectomized hosts will have to verify this notion.

The Ag used in the present and previous studies (16, 19) to demonstrate the existence of extrathymically determined self-K/D–restricted CTL in nude mice is TNP. It could be argued that the nude mouse extrathymic T cell repertoire is unusually restricted in exhibiting only TNP-specific responses, and that our data therefore do not allow the general conclusion that young nude mice possess class I– but lack class II–restricted T cell responses. The following obervations make this possibility unlikely. First, a recent study by Melief and coworkers[2] demonstrates that nude mice have extrathymically determined K region–restricted, Sendai virus–specific T cells. Thus, in two antigenically different systems, TNP and Sendai, nude mice exhibit class I–restricted T cells. Since nude mice also have easily demonstrable alloreactive CTL precursors and since most allorecognition reflects self plus nominal Ag cross-reactive recognition, it is a matter of time until other nominal Ag are found that the nude mouse T cells can recognize.

[2] W. M. Kast, L. P. de Waal, and C. M. Melief. 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. Manuscript submitted for publication.

Second, it should be noted that for both TNP (this report) and Sendai virus,[2] only class I–restricted T cells and no class II–restricted T cells were found in the nude mouse. Together with the observation that in the present system, no class II–restricted T cells to a variety of protein Ag (i.e., cyto *c*, TGAL, GLϕ, collagen, and PPD) could be detected in nude mice, we interpret our findings as reflecting the presence of class I–restricted and absence of class II–restricted T cells in nude mice.

If one accepts the concept that, in nude mice, CTL committed to self-K/D recognition exist while T cells committed to I region recognition are absent, the question arises as to how the nude mouse environment provides for education of K/D-specific T cells but not for I region–specific T cells. An attractive possibility is provided by the observation of Jenkinson et al. (35), who compared the expression of MHC antigens on mesenchymal and epithelial cells in the developing normal and nude mouse thymus. They found that the embryonic nude thymus, although comparable to the normal in its expression of K region MHG antigens, completely lacks demonstrable Ia antigens at any time during development. This failure of Ia expression might reflect either a defect in or a total absence of those cells that normally express Ia. In either case, one could speculate that, as education for I region–restricted T cells seems to be a process strictly dependent on intrathymic interactions (9–13), the nude mouse fails to develop any I region–specific T cells. In contrast, prenatal interaction of lymphoid cells with the K region–bearing epithelial cells in the nude mouse thymic rudiment could conceivably lead to education of K and presumably also D region–restricted T cells such as CTL. The functional ability of the embryonic thymus in terms of imposing restriction patterns is illustrated by the finding of MHC-restricted CTL in the fetal liver by day 18 (36). Thus, the so-called extrathymic CTL repertoire in nude mice could very well be in fact intrathymically derived during fetal development. Alternatively, the extrathymic CTL repertoire may be a consequence of the interaction of precursor cells with extrathymic K/D-bearing cells at unknown sites in the nude environment that do not allow for education of I region–restricted T cells.

The question of the origin of the CTL specific for extrathymic K/D antigens in radiation-induced BM chimeras in which the donor is a normal mouse may be a separate issue (12–15). One explanation could be that, in chimeras, the extrathymic repertoire is due to education on donor BM–derived, K/D-bearing elements in the chimera, is a mechanism through which part of the nude mouse extrathymic CTL repertoire may be generated as well, in addition to its intrathymic pathway of K/D region commitment. Alternatively, the CTL restricted to extrathymic K/D antigens in chimeras could be descendants from Thy-1-negative but already K/D-committed (through intrathymic traffic) T cells in the BM that have escaped the usual T cell depletion procedures applied to BM before its use in reconstitution (37). This would explain the extrathymic CTL repertoire observed in both "normal" $F_1 \rightarrow$ parent chimeras as well as nude $F_1 \rightarrow$ parent chimeras: K/D region–committed, Thy-1-negative or low CTL precursors educated in either the normal F_1 thymus or the nude mouse thymus rudiment (expressing K/D region MHC antigens) or extrathymic environment might undergo further maturation and expansion in the irradiated parental host

without being further influenced in their MHC restriction pattern. However, one problem with this hypothesis is that, in order to explain the lack of an extrathymic I region–restricted repertoire in chimeras, one would have to assume that the donor BM does not contain Thy-1-negative, I region–committed T cells. Therefore, we favor the first hypothesis. Whatever mechanism is correct, the extrathymic CTL repertoire of chimeras and nude mice should, until further comparisons are made, not be regarded as identical in origin and/or nature.

The strict dependency of I region–restricted cells on intrathymic differentiation suggests the existence of a unique, thymus-associated, Ia-positive cell or a uniquely intrathymic interaction between precursor T cells and Ia-positive elements that cannot be provided anywhere else in the environment in which T cells differentiate. It is now clear (38, 39) that T cell I region recognition is acquired in the thymus through interaction with a BM-derived cell that functions as an APC. Studies on the thymus from chimeras indicate that medullary Ia-positive cells are donor BM derived (40) and of the dendritic or interdigitating type,[3] while corticol Ia-positive epithelial cells are of the host type (40). Thus, I region restriction most likely results from intrathymic interactions between noncommitted T cells and medullary Ia-positive cells. Studies in neonatally anti-Ia-treated mice indicate a correlation between a decrease in thymic Ia-Ag expression, a decrease in the development of Ia-specific T cells (41), and a decrease in thymic APC function for I region–specific T cells (Kruisbeek and Longo, manuscript in preparation). This model will be used to further identify the thymic Ia-positive cells responsible for "education" of I region–specific T cells, possibly through the use of specific monoclonal Ab to thymic stromal cells (42).

In conclusion, the present results demonstrate that nude mice that do have CTL with self-specificity for K/D region determinants, lack proliferating T cells with self-specificity for I region determinants. It was previously suggested on the basis of studies with radiation-induced BM chimeras that education for I region restriction is stictly an intrathymically determined event (12, 13). The present study provides further evidence for this concept by demonstrating that young athymic nude mice lack I region–specific proliferating T cells, supporting the notion that nude mice lack the unique thymic elements responsible for education of I region–restricted T cells. The generation of CTL with self-specificity for K/D region Ag in nude mice may either be intrathymic, through interaction with K/D region–expressing elements in the rudimentary nude mouse thymus, or extrathymic, through interaction with as yet undefined extrathymic elements. The latter mechanism is most likely responsible for the determination of the self-specificity expressed by extrathymic CTL in chimeras. The present data are also consistent with the notion that the self-specificity of Ia- and K/D-rectricted T cells is determined at least in part independently on different host restriction elements.

[3] Duyvestyn, A. M., and A. N. Barclay. Identification of the bone marrow derived Ia positive cells in the rat thymus. A morphological and cytochemical study. Manuscript submitted for publication.

Summary

The presence in athymic nude mice of precursor T cells with self-recognition specificity for either H-2 K/D or H-2 I region determinants was investigated. Chimeras were constructed of lethally irradiated parental mice receiving a mixture of F_1 nude mouse (6–8 wk old) spleen and bone marrow cells. The donor inoculum was deliberately not subjected to any T cell depletion procedure, so that any potential major histocompatibility complex–committed precursor T cells were allowed to differentiate and expand in the normal parental recipients. 3 mo after reconstitution, the chimeras were immunized with several protein antigens in complete Freund's adjuvant in the footpads and their purified draining lymph node T cells tested 10 d later for ability to recognize antigen on antigen-presenting cells of either parental haplotype. Also, their spleen and lymph node cells were tested for ability to generate a cytotoxic T lymphocyte (CTL) response to trinitrophenyl (TNP)-modified stimulator cells of either parental haplotype. It was demonstrated that T cell proliferative responses of these F_1(nude) → parent chimeras were restricted solely to recognizing parental host I region determinants as self and expressed the Ir gene phenotype of the host. In contrast, CTL responses could be generated (in the presence of interleukin 2) to TNP-modified stimulator cells of either parental haplotype. Thus these results indicate that nude mice which do have CTL with self-specificity for K/D region determinants lack proliferating T cells with self-specificity for I region determinants. These results provide evidence for the concepts that development of the I region–restricted T cell repertoire is strictly an intrathymically determined event and that young nude mice lack the unique thymic elements responsible for education of I region–restricted T cells.

We would like to thank Carl Hansen and Damara Bolte for constructing and raising the F_1 nude mice, Louise Pastuck for typing the manuscript, and Richard J. Hodes and Alfred Singer for help in reviewing the manuscript.

Received for publication 23 March 1984 and in revised form 31 May 1984.

References

1. Bevan, M. J. 1977. In a radiation chimera, host H-2 antigens determine immune responsiveness of donor cytotoxic cells. *Nature* (*Lond.*). 269:417.
2. Sprent, J. 1978. Restricted helper function of F_1 hybrid T cells positively selected to heterologous erythrocytes in irradiated parental strain mice. I. Evidence for restrictions affecting helper cell induction and T-B collaboration, both mapping to the K-end of the H-2 complex. *J. Exp. Med.* 147:1159.
3. Kappler, J. W., and P. Marrack. 1978. The role of H-2-linked genes in helper T cell function. IV. The importance of T cell genotype and host environment in I region and Ir-gene expression. *J. Exp. Med.* 148:1510.
4. Zinkernagel, R. M., G. N. Callahan, J. Klein, and G. Dennert. 1978. Cytotoxic T cells learn specificity for self H-2 during differentiation in the thymus. *Nature* (*Lond.*). 271:251.
5. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, P. A. Klein, and J. Klein. 1978. On the thymus in the differentation of "H-2 self-recognition" by T cells: evidence for dual recognition? *J. Exp. Med.* 147:882.

6. Fink, P. J., and M. J. Bevan. 1978. H-2 antigens of the thymus determine lymphocyte specificity. *J. Exp. Med.* 148:766.
7. Singer, A., K. S. Hathcock, and R. J. Hodes. 1981. Self-recognition in allogeneic radiation bone marrow chimeras. A radiation-resistant host element dictates the self-specificity and immune response gene phenotype of T-helper cells. *J. Exp. Med.* 153:1286.
8. Longo, D. L., and R.H. Schwartz. 1981. Inhibition of antigen-induced T cells from radiation-induced bone marrow chimeras by a monoclonal antibody directed against an Ia determinant on the antigen-presenting cell. *Proc. Natl. Acad. Sci. USA.* 78:514.
9. Singer, A., K. S. Hathcock, and R. J. Hodes. 1982. Self-recognition in allogeneic thymic chimeras. Self-recognition by T-helper cells from thymus engrafted nude mice is restricted to the thymic H-2 haplotype. *J. Exp. Med.* 155:339.
10. Hedrick, S. M., and J. Watson. 1979. Genetic control of the immune response to collagen. II. Antibody responses produced in fetal liver restored radiation chimeras and thymus-reconstituted F_1 nude mice. *J. Exp. Med.* 150:646.
11. Glimcher, L. H., R. H. Schwartz, D. L. Longo, and A. Singer. 1982. The specificity of the syngeneic mixed lymphocyte response, a primary anti-I-region T cell proliferative response, is determined intrathymically. *J. Immunol.* 129:987.
12. Bradley, S. M., A. M. Kruisbeek, and A. Singer. 1982. Cytotoxic T lymphocyte responses in allogeneic radiation bone marrow chimeras. The chimeric host strictly dictates the self-repertoire of Ia-restricted T cells but not H-2 K/D restricted T cells. *J. Exp. Med.* 156:1650.
13. Kruisbeek, A. M., S. O. Sharrow, and A. Singer. 1983. Differences in the MHC restricted self-recognition repertoire of intra-thymic and extra-thymic cytotoxic T lymphocyte precursors. *J. Immunol.* 130:1027.
14. Wagner, H., C. Hardt, H. Stockinger, K. Pfizenmaier, R. Bartlet, and M. Rollinghoff. 1981. The impact of the thymus on the generation of immunocompetence and diversity of antigen-specific MHC-restricted cytotoxic T-lymphocyte precursors. *Immunol. Rev.* 58:95.
15. Doherty, P. C., R. Korngold, D. H. Schwartz, and J. R. Bennink. 1981. The development and loss of virus-specific thymic competence in bone marrow radiation chimeras and normal mice. *Immunol. Rev.* 58:37.
16. Kruisbeek, A. M., S. O. Sharrow, B. J. Mathieson, and A. Singer. 1981. The H-2 phenotype of the thymus dictates the self-specificity expressed by thymic but not splenic cytotoxic T lymphocyte precursors in thymus-engrafted nude mice. *J. Immunol.* 127:2168.
17. Kruisbeek, A. M., P. M. Andrysiak, and A. Singer. 1983. Self-recognition of accessory cell Ia determinants is required for the in vitro generation of hapten-specific cytotoxic T lymphocyte responses. *J. Immunol.* 131:1650.
18. Gillis, S., N. A. Union, P. E. Baker, and K. A. Smith. 1979. The *in vitro* generation and sustained culture of nude mouse cytotoxic T lymphocytes. *J. Exp. Med.* 149:1460.
19. Hunig, T., and M. J. Bevan. 1980. Specificity of cytotoxic T cells from athymic mice. *J. Exp. Med.* 152:688.
20. Maryanski, J. L., H. R. MacDonald, B. Sordat, and J. C. Cerottini. 1981. Cytotoxic T lymphocyte precursor cells in congenitally thymic C57BL/6 nu/nu mice: quantitation, enrichment and specificity. *J. Immunol.* 126:871.
21. Ando, I., and M. Hurme. 1981. Self-MHC restricted cytotoxic T cell responses without thymic influence. *Nature (Lond.).* 289:494.
22. Longo, D. L., and R. H. Schwartz. 1980. Gene complementation. Neither *Ir*-GLϕ gene need be present in the proliferative T cell to generate an immune response to poly(Glu^{55}Lys^{36}Phe^{9}). *J. Exp. Med.* 151:1452.

23. Ozato, K., N. Mayer, and D. H. Sachs. 1980. Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. *J. Immunol.* 124:533.
24. Ozato, K., and D. H. Sachs. 1981. Monoclonal antibodies to mouse MHC antigens. III. Hybridoma antibodies reacting to antigens of the H-2^b haplotype reveal genetic control of isotype expression. *J. Immunol.* 126:317.
25. Corradin, G., H. M. Ettinger, and J. M. Chiller. 1977. Lymphocyte specificity to protein antigens. I. Characterization of the antigen-induced *in vitro* T cell dependent proliferative response with lymph node cells from primed mice. *J. Immunol.* 119:1048.
26. Kappler, J. W., B. Skidmore, J. White, and P. Marrack. 1981. Antigen-inducible, *H-2*-restricted, interleukin-2-producing T cell hybridomas. Lack of independent antigen and *H-2* restriction. *J. Exp. Med.* 153:1198.
27. Click, R. E., L. Benck, and B. J. Alter. 1972. Immune responses in vitro. I. Culture conditions for antibody synthesis. *Cell. Immunol.* 3:264.
28. Shearer, G. M. 1974. Cell-mediated cytotoxicity to trinitrophenyl modified syngeneic cells. *Eur. J. Immunol.* 4:527.
29. Billings, P., S. J. Burakoff, M. E. Dorf, and B. Benacerraf. 1978. Genetic control of cytotoxic T lymphocyte responses. I. Ir-gene control of the specificity of cytotoxic T lymphocyte responses to trinitrophenyl-modified syngeneic cells. *J. Exp. Med.* 148:341.
30. Shearer, G. M., A. M. Schmidt-Verhulst, C. B. Pettinelli, M. W. Miller, and P. E. Gilheany. 1979. H-2 inbred genetic control of murine T cell mediated lympholysis to autologous cells modified with low concentrations of trinitrobenezene sulfonate. *J. Exp. Med.* 149:1407.
31. MacDonald, H. R., R. K. Lees, A. L. Glasebrook, and B. Sordat. 1982. Interleukin 2 production by lymphoid cells from congenitally athymic (nu/nu) mice. *J. Immunol.* 129:251.
32. Klein, J. R., and M. J. Bevan. 1983. Secretion of immune interferon and generation of cytotoxic T cell activity in nude mice are dependent on interleukin 2: age-associated endogenous production of interleukin 2 in nude mice. *J. Immunol.* 130:1780.
33. MacDonald, H. R., and R. K. Lees. 1984. Frequency and specificity of precursors of interleukin 2-producing cells in nude mice. *J. Immunol.* 132:605.
34. Kronke, M., P. Scheurich, K. Pfizenmaier, M. Rollinghoff, and H. Wagner. 1982. T cell interactions during in vitro cytotoxic T lymphocyte responses. V. Precursor frequencies and specificity of alloreactive helper T cells. *J. Exp. Med.* 156:41.
35. Jenkinson, E. J., W. van Ewijk, and J. J. T. Owen. 1981. Major histocompatibility complex antigen expression on the epithelium of the developing thymus in normal and nude mice. *J. Exp. Med.* 153:280.
36. Galli, P., and W. Droege. 1980. Development of cytotoxic T lymphocyte precursors in the absence of the thymus. *Eur. J. Immunol.* 10:87.
37. Duprez, V., B. Hamilton, and S. J. Burakoff. 1982. Generation of cytotoxic T lymphocytes in thymectomized irradiated and bone marrow-reconstituted mice. *J. Exp. Med.* 156:844.
38. Longo, D. L., and R. H. Schwartz. 1980. T-cell specificity for H-2 and Ir-gene phenotype correlates with the phenotype of thymic antigen-presenting cells. *Nature (Lond.).* 287:44.
39. Longo, D. L., and M. L. Davis. 1983. Early appearance of donor-type antigen presenting cells in the thymuses of 1200 R radiation-induced bone marrow chimeras correlates with self-recognition of donor I region gene products. *J. Immunol.* 130:2525.
40. Barclay, A. N., and G. Mayrhofer. 1981. Bone marrow origin of Ia-region positive cells in the medulla of rat thymus. *J. Exp. Med.* 153:1666.

41. Kruisbeek, A. M., M. J. Fultz, S. O. Sharrow, A. Singer, and J. J. Mond. 1983. Early development of the T cell repertoire. In vivo treatment of neonatal mice with anti-Ia antibodies interferes with differentiation of I-restricted T cells but not K/D-restricted T cells. *J. Exp. Med.* 157:1932.
42. Van Vliet, E., M. Melis, and W. van Ewijk. 1984. Monoclonal antibodies to stromal cell types of the mouse thymus. *Eur. J. Immunol.* In press.