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

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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-recogni-
tion 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

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\(^1\) 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; Glu\(^5\)Lys\(^3\)Phe, poly(Glu\(^5\)Lys\(^3\)Phe); IL-2,
interleukin 2; LN, lymph node; MHC, major histocompatibility complex; PBS, phosphate-buffered
saline; PPD, purified protein derivative of Mycobacterium tuberculosis; TNP, trinitrophenyl.
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 F1 nude mouse (6–8 wk old) splen 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 F1 (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)F1, (B10 × B10.D2)F1, (C3H × B6)F1 (nude), and (B10 Scn × B10.D2)F1 (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 137Cs source at 128 rad/min and were reconstituted within 6 h with 107 F1 BM cells along with (in some experiments) 2 × 107 F1 spleen cells administered intravenously. The F1 cells were from donors of the following kind: (a) normal F1 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) F1 nude heterozygotes that are phenotypically normal and not T cell depleted in vivo or in vitro; (c) F1 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-KkD k plus C [for (C3H × B6)F1 → B10 chimeras], anti-Kd plus C [for (B10 × B10.D2)F1 → B10 chimeras], or anti-KbD b plus C [for (B10.D2 × B10)F1 → B10.D2 chimeras]. Such treatment always completely removed any T cell function, indicating that the responding cells were of donor F1 origin. Monoclonal Ab used for this treatment were: 15-1-5P (anti-
K₄D₄, cross-reactive on D₄a-q) (23); 15-5-5S (anti-D₄, cross-reactive with K₄D₄) (23); and 28-8-6S (anti-K₄D₄) (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(Glu₅₆Lys₃₅Phe₉)n (GL₄₉) (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 H37Ra.

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 X B10.D2)F₁—→ parent mice by passage over nylon wool columns (25) followed by anti-I-A plus C treatment. Nylon-passed cells were adjusted to 10 × 10⁶ cells/ml in medium containing 1 µg/ml of MK-D6 protein A—purified Ab (anti-I-A α) (26) and incubated at 4°C for 45 min. After one wash, the cells were resuspended at 10 × 10⁶ 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 × 10⁶/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. 10⁷ 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 × 10⁶ LN cells plus soluble Ag; 4 × 10⁶ LN T cells plus 2 × 10⁶ nonpulsed spleen cells plus soluble Ag; or 4 × 10⁶ LN T cells plus 2 × 10⁹ 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, penicillin (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 [³H]thymidine (6.7 Ci/mM) (New England Nuclear, Boston, MA) was added per well and the cultures were harvested 16–18 h later. [³H]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 × 10⁶ splenic or LN responder cells and 2 × 10⁶ 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 ⁵¹Cr-release assay was performed on day 5 or 6 with TNP-modified, ⁵¹Cr-labeled, Con A—induced splenic blasts as target cells. The percent specific ⁵¹Cr release = 100 × [(experimental — spontaneous release)/(detergent — spontaneous release)]. Data shown are the means of triplicate determinations using 5 × 10⁵ target cells (SD < 8%) and are representative of at least five separate experiments. Specific ⁵¹Cr release from unmodified targets was always <4%. Maximum ⁵¹Cr 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-mann
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 F₁(Nude) → Parent and F₁(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 F₁ nude mice or from their F₁ heterozygous (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 F₁ nude mouse cells with those of recipients of T cell-depleted normal F₁(nude/+) cells. As shown in Table II, a PPD response occurred in (C3H × B6) F₁ → B6 chimeras, regardless of whether the donor inoculum consisted of F₁ nude BM or T cell-depleted F₁(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-2Kk 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**

<table>
<thead>
<tr>
<th>Strain</th>
<th>H-2</th>
<th>PPD</th>
<th>TGAL</th>
<th>GL4</th>
<th>Collagen</th>
<th>Pigeon cyto c</th>
</tr>
</thead>
<tbody>
<tr>
<td>B10, B6</td>
<td>b</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C3H</td>
<td>k</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>B10.D2</td>
<td>d</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B10.A</td>
<td>a</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>(B6 × C3H)F₁</td>
<td>(b × k)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(B10 × B10.D2)F₁</td>
<td>(b × d)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>


## Table II
Comparison of the T Cell Proliferative Responses in F₁ → Parent Chimeras Receiving Either F₁(Nude) or T Cell-depleted F₁(Nude/+ ) Donor Cells

<table>
<thead>
<tr>
<th>LN Cells*</th>
<th>Antigens $^d$</th>
<th>Donor $^a$ →Recipient</th>
<th>None</th>
<th>PPD</th>
<th>TGAL</th>
<th>GLφ</th>
<th>Pigeon cyto c</th>
</tr>
</thead>
<tbody>
<tr>
<td>(B6 × C3H)F₁(nude)(BM) →B6</td>
<td>1.277 ± 170</td>
<td>48.270 ± 3.790</td>
<td>81.555 ± 4.953</td>
<td>1.845 ± 446</td>
<td>1.566 ± 170</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B6 × C3H)F₁(nude) (BM plus spleen) →B6</td>
<td>3.058 ± 1.323</td>
<td>83.252 ± 1.784</td>
<td>150.024 ± 6.440</td>
<td>2.236 ± 867</td>
<td>1.992 ± 444</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B6 × C3H)F₁(nude/+)(T-depleted BM) →B6</td>
<td>1.298 ± 718</td>
<td>67.368 ± 5.092</td>
<td>59.772 ± 7.561</td>
<td>1.358 ± 236</td>
<td>1.509 ± 587</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B6 × C3H)F₁(nude/+)(T-depleted BM plus spleen) →B6</td>
<td>3.454 ± 864</td>
<td>102.452 ± 4.066</td>
<td>152.434 ± 8.211</td>
<td>1.658 ± 394</td>
<td>1.796 ± 282</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B6 × C3H)F₁(nude/+)(Control)</td>
<td>2.408 ± 219</td>
<td>103.472 ± 6.731</td>
<td>96.9731 ± 7.127</td>
<td>51.357 ± 2.818</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 F₁ BM rigorously depleted of contaminating T cells, this result is to be expected (22). However, in F₁ → parent chimeras made with F₁ 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)F₁ → B10 and (B10 × B10.D2)F₁ → B10.D2 chimeras, using either nude mice or nude/+ normal litter mates as donors. The donor inoculum from the nude/+ F₁ mice was in these experiments used without any preceding T cell depletion procedure. In Table III it can be seen that in chimeras made with F₁(nude/+ ) BM or BM plus spleen, no host restriction was observed: in F₁ → B10 chimeras, responses to GLφ were observed despite the fact that the host is a nonresponder; in F₁ → B10.D2 chimeras, collagen responses were observed, despite the nonresponder phenotype of the host. In contrast, the chimeras made with F₁(nude) BM or BM plus spleen strictly displayed the host Ir gene phenotype: positive collagen and negative GLφ responses in F₁(nude) → B10 chimeras and positive GLφ and negative collagen responses in F₁(nude) → B10.D2 chimeras. All chimeras generated full responses to PPD, an Ag to which both parental haplotypes are responders. Treatment of the F₁ → B10 chimeric T cells with anti-H-2K$^d$ plus C and of F₁ → B10.D2 chimeric T cells with anti-H-2K$^d$D$^b$ 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 F₁(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 F1(Nude) → Parent Chimeras, But Not from Untreated F1(Nude/+ → Parent Chimeras, Display the Ir Gene Phenotype of the Host**

<table>
<thead>
<tr>
<th>LN cells*</th>
<th>Antigen+</th>
<th>(B10 × B10.D2) donor -+</th>
<th>Recipient</th>
<th>None</th>
<th>GLφ</th>
<th>Collagen</th>
<th>PPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Nude/+ BM → B10</td>
<td>1,209 ± 271</td>
<td>88,655 ± 5,102</td>
<td>57,856 ± 4,340</td>
<td>157,819 ± 11,220</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Nude/+ BM plus spleen → B10</td>
<td>1,617 ± 311</td>
<td>73,368 ± 8,189</td>
<td>43,771 ± 2,056</td>
<td>118,252 ± 9,993</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Nude/+ BM → B10.D2</td>
<td>2,529 ± 629</td>
<td>79,955 ± 6,019</td>
<td>30,350 ± 2,941</td>
<td>104,383 ± 11,519</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nude/+ BM plus spleen → B10.D2</td>
<td>1,961 ± 215</td>
<td>90,424 ± 5,212</td>
<td>39,789 ± 5,212</td>
<td>126,511 ± 12,959</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Nude) BM → B10</td>
<td>3,125 ± 455</td>
<td>3,044 ± 511</td>
<td>28,951 ± 511</td>
<td>92,886 ± 8,178</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Nude) BM plus spleen → B10</td>
<td>1,389 ± 550</td>
<td>1,456 ± 515</td>
<td>40,079 ± 2,829</td>
<td>110,629 ± 12,689</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Nude) BM → B10.D2</td>
<td>2,888 ± 550</td>
<td>67,488 ± 2,933</td>
<td>3,008 ± 268</td>
<td>155,588 ± 18,770</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Nude) BM plus spleen → B10.D2</td>
<td>2,014 ± 367</td>
<td>90,521 ± 11,204</td>
<td>2,621 ± 119</td>
<td>140,020 ± 9,191</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* See footnote * to Table II.
+ See footnote † to Table II.
† In contrast to Table II, the F1(nude/+ donor inoculum had not been subjected to any T cell depletion procedures.

### TABLE IV

**Proliferating T Cells From F1(Nude BM plus Nude Spleen) → Parent Chimeras But Not From F1(Nude BM plus Nude/+ Spleen) → Parent Chimeras Display the Ir Gene Phenotype of the Host**

<table>
<thead>
<tr>
<th>LN cells*</th>
<th>Antigen+</th>
<th>(B6 × C57Fl(nude BM) → B10.A</th>
<th>Recipient</th>
<th>None</th>
<th>PPD</th>
<th>TGAL</th>
<th>GLφ</th>
<th>Pigeon cyto c</th>
</tr>
</thead>
<tbody>
<tr>
<td>(B6 × C57Fl(nude BM) → B10.A</td>
<td>1,188 ± 211</td>
<td>75,858 ± 6,470</td>
<td>1,156 ± 195</td>
<td>1,229 ± 501</td>
<td>17,256 ± 1,410</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B6 × C57Fl(nude BM plus nude spleen) → B10.A</td>
<td>1,855 ± 318</td>
<td>50,817 ± 4,237</td>
<td>1,988 ± 374</td>
<td>2,021 ± 258</td>
<td>11,882 ± 5,152</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B6 × C57Fl(nude BM plus nude/+ spleen) → B10.A</td>
<td>2,036 ± 406</td>
<td>102,119 ± 11,514</td>
<td>81,129 ± 7,129</td>
<td>56,774 ± 6,312</td>
<td>23,314 ± 5,489</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* See footnote * to Table II.
† See footnote † to Table II.

F1 nude spleen, these could have been expanded to detectable levels. To address this issue, an experiment was performed in which chimeras were made with F1 nude BM (shown above to contain no I region-committed T cells; see Tables II, III) supplemented with either F1 nude spleen or F1(nude/+ spleen). As shown in Table IV, the addition of F1 nude spleen to F1 nude BM did not affect the results: the F1 → 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 F1(nude/+ spleen cells were added to the F1(nude) BM, the chimeras displayed the Ir gene phenotype of the F1 donor, i.e., responsiveness to GLφ, (T,G)—A—L, and pigeon cyto c. Thus, these experiments document that I region-committed F1(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 F1(nude) BM. We therefore conclude that the inability of F1(nude) BM plus...
PPD-specific T Cells from F1(Nude) → Parent Chimeras, But Not from Untreated F1(Nude/+) → Parent Chimeras, Are Restricted to Self-recognizing I-A Ag of the Host

<table>
<thead>
<tr>
<th>LN T cells*</th>
<th>Monoclonal Ab specificity†</th>
<th>Antigen-presenting cells‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>(B10 × B10.D2) donor untreated BM plus spleen → Recipient</td>
<td>B10</td>
<td>PPD-pulsed B10</td>
</tr>
<tr>
<td>Nude/+ → B10</td>
<td>None</td>
<td>1,047 ± 189</td>
</tr>
<tr>
<td></td>
<td>1-A&lt;sub&gt;b&lt;/sub&gt;</td>
<td>21,020 ± 1,919</td>
</tr>
<tr>
<td></td>
<td>1-A&lt;sub&gt;d&lt;/sub&gt;</td>
<td>77,488 ± 10,172</td>
</tr>
<tr>
<td>Nude/+ → B10.D2</td>
<td>None</td>
<td>2,211 ± 794</td>
</tr>
<tr>
<td></td>
<td>1-A&lt;sub&gt;b&lt;/sub&gt;</td>
<td>17,251 ± 2,611</td>
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<td></td>
<td>1-A&lt;sub&gt;d&lt;/sub&gt;</td>
<td>92,581 ± 7,884</td>
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<tr>
<td>Nude → B10</td>
<td>None</td>
<td>1,556 ± 99</td>
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<td></td>
<td>1-A&lt;sub&gt;b&lt;/sub&gt;</td>
<td>8,286 ± 324</td>
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<td></td>
<td>1-A&lt;sub&gt;d&lt;/sub&gt;</td>
<td>50,886 ± 1,297</td>
</tr>
<tr>
<td>Nude → B10.D2</td>
<td>None</td>
<td>327 ± 10</td>
</tr>
<tr>
<td></td>
<td>1-A&lt;sub&gt;b&lt;/sub&gt;</td>
<td>1,758 ± 186</td>
</tr>
<tr>
<td></td>
<td>1-A&lt;sub&gt;d&lt;/sub&gt;</td>
<td>1,810 ± 71</td>
</tr>
</tbody>
</table>

* 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<sup>5</sup>/0.2 ml culture for 4 d. The donor cells had not been subjected to any T cell depletion procedure.
† Protein A-purified Abs were used at 5 μg/ml, added at beginning of the culture period. Anti-I-A<sub>b</sub>: 25-9-3S (24); anti-I-A<sub>d</sub>: 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<sup>5</sup> per culture. Without APC, the response of purified LN T cells to soluble PPD was <2,100 cpm in each experimental group.

We next investigated whether F1(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 F1(nude) → B10 and F1(nude) → B10.D2 chimeras. However, when donor F<sub>1</sub> 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<sub>1</sub>(nude) → B10 T cells only responded to PPD-pulsed B10 spleen and F<sub>1</sub>(nude) → B10.D2 T cells only responded to PPD-pulsed B10.D2 spleen (Table V). Thus, the genotypic F<sub>1</sub>(nude) T cells had become restricted to recognition of host MHC determinants. Not surprisingly, the F<sub>1</sub>(nude/+) → parent chimeras failed to demonstrate such host restriction: T cells from either F<sub>1</sub>(nude/+) → B10 or F<sub>1</sub>(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<sub>b</sub> and on B10.D2 spleen with anti-I-A<sub>d</sub> (Table V) demonstrated that these responses were I region restricted.

Finally, the H-2 restriction of the I<sub>d</sub>r gene-controlled responses to GL<sub>φ</sub> and collagen were tested. T cells isolated from F<sub>1</sub> → B10 and F<sub>1</sub> → B10.D2 chimeras were tested for their ability to respond to GL<sub>φ</sub>-pulsed or collagen-pulsed spleen cells from either parental haplotype. B10 recipients of F<sub>1</sub>(nude) BM plus spleen only responded to collagen-pulsed responder B10 spleen and not to GL<sub>φ</sub>-pulsed...
spleen of either parental haplotype (Table VI). In the reciprocal Fl(nude) → B10.D2 combination, only responses to GL4-pulsed responder B10.D2 spleen were observed. In contrast, Fl(nude/+) → B10 chimeras and Fl(nude/+) → B10.D2 chimeras both responded equally well to GL4-pulsed B10.D2 and collagen-pulsed B10 spleen (Table VI), indicating that I region-committed Fl T cells are not affected (negatively selected, suppressed, or restricted) by developing in the parental host environment.

In conclusion, chimeric recipients of Fl(nude) BM-plus-spleen generated T cells with I region specificity and Ir gene phenotype of the irradiated host, while recipients of Fl(nude/+) BM plus spleen (when not T cell depleted) generated T cells indistinguishable from the Fl 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 Fl(Nude) → Parent and Fl(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 Fl(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., Fl(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 Fl(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 Fl(nude) → parent chimeras (same mice as used in Table III) are restricted to recognizing TNP in association
with host MHC determinants: $F_1(nude) \rightarrow B10$ chimeras only responded to TNP-modified B10 stimulators, while $F_1(nude) \rightarrow B10.D2$ chimeras were only stimulated by TNP-modified B10.D2 stimulators. Identical results were obtained with LN cell CTL responses (data not shown). In contrast, $F_1 \rightarrow parent$ 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 $F_1(nude) \rightarrow parent$ 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 F1 (nude) → parent chimeras in the presence and absence of exogenous helper factors. Spleen cells from chimeras made with a mixture of 2 × 10^5 spleen cells and 1 × 10^5 BM cells from nude (B10 × B10.D2)F1 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-o-mannoside). Specific ^{31}Cr-release values represent the means of triplicate determinations (SD < 7%) and are representative of six separate experiments with individual mice. Absolute ^{31}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). ^{31}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 F1 (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, F1 (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 F1 (nude) mice, it is important to determine whether the non-host-restricted CTL from F1 (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 F1 (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)F1(Nude) → Parent Chimeras in the Absence and Presence of Con A SN

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<tr>
<td>(B10 × B10.D2) donor untreated BM → Recipient</td>
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<tr>
<td>Nude → B10</td>
<td>-</td>
<td>20</td>
<td>B10-TNP</td>
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<td>13</td>
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<td>-2</td>
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<td>54</td>
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<td></td>
<td>19</td>
<td>0</td>
<td>-3</td>
<td>2</td>
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<td>-</td>
<td>20</td>
<td>B10-TNP</td>
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<td>0</td>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td>Nude → B10.D2</td>
<td>+</td>
<td>20</td>
<td>B10.D2-TNP</td>
<td>54</td>
<td>13</td>
<td>20</td>
<td>82</td>
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<td></td>
<td>31</td>
<td>5</td>
<td>10</td>
<td>44</td>
</tr>
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</table>

* Values represent the percent of specific ¹¹⁶Cr release of the indicated target cells (means of triplicate determinations; SD < 5%). The percent of specific ¹¹⁶Cr release of target cells not modified with TNP was <4% in all groups.

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 F1(nude) → parent chimeras. The F1 CTL in the spleen and LN of F1(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 F1(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 \( F_1(nude/+)(untreated) \rightarrow \) 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 \( F_1(nude) \rightarrow \) 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 \( \rightarrow \) 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 \( F_1 \rightarrow \) 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 \( F_1(nude) \rightarrow \) parent chimeras (present experiments) or by an allogeneic thymus graft (9, 11). \( F_1(nude) \rightarrow \) parent chimeras are indistinguishable from \( F_1(nude/+)(T \) depleted) \( \rightarrow \) 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 F1(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 F1 → parent chimeras made with T cell-depleted normal F1 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 F1(nude) → parent and normal F1 → 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 F1(nude) → parent chimeras are the descendants of the self-H-2-committed CTL precursors present in the F1(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 F1(nude) → parent chimeric host or before that in the F1(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 observations make this possibility unlikely. First, a recent study by Melief and coworkers 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, 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” F₁ → parent chimeras as well as nude F₁ → parent chimeras: K/D region-committed, Thy-1-negative or low CTL precursors educated in either the normal F₁ 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, while cortical 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 strictly 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-restricted T cells is determined at least in part independently on different host restriction elements.

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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 F1 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 F1(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.

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