Tpre, A NEW ALLOANTIGEN ENCODED IN THE IgT-C REGION OF CHROMOSOME 12, IS EXPRESSED ON BONE MARROW OF NUDE MICE, FETAL T CELL HYBRIDS, AND FETAL THYMUS*

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A cluster of T cell-specific alloantigens, encoded by genes linked to Igh-1, have been hypothesized to be constant region determinants for T cell receptors in the mouse (1–4). Similar antigens have been shown to be present on antigen-specific T cell factors (5, 6). It has been shown that three of these structural products (Tthy, Tind, and Tsu) are distributed on cells in an ordered sequence during maturation and ontogeny (4). All of these determinants are, however, expressed on T cells postnatally and do not appear in the nude animal (4), a well-documented source of pre-T cells (7). It is known that T cells can be sensitized to respond to alloantigens in an antigen-specific pattern after 14 d in organ culture (8) and that the restriction to self-recognition and allorecognition occurs before thymic trafficking (9). We therefore conclude that cells must express antigen receptors at the pre-T cell level. This rationale led to the search for a pre-T cell antigen receptor. This paper describes an alloantigen on prethymic T cells that is genetically linked in expression to the region proposed to be IgT-C.

Fetal T cell hybrids, which were negative for the Tthy, Tind, and Tsu antigens, were used as a target to screen monoclonal antibodies generated in outbred nu/nu (Igh-1^a) animals immune to C.AL-20 (Igh-1^d) 17-d fetal thymocytes. This path led to the selection of F.6.9.1, a μk monoclonal anti-Tpre^d. The alloantigen recognized is expressed on fetal T cells, some adult thymocytes, adult bone marrow, and spleen (in low frequency or density). The homozygous nu^{str}/nu^{str} bone marrow is a rich source of this cell, in contrast to the cells expressing Tthy, Tind, and Tsu, which fail to appear in the nude animal. An arrest in maturation in the nude animal may result in failure of this cell to exit from the marrow in high numbers.

Methods

Generation of Fetal Thymocyte, Fetal T Liver, and Fetal B Liver Hybrids. Fetal thymocytes, or fetal liver cells, were removed from developing mice by dissection under low-power microscopy. The time of gestation was estimated from the morphology of the fetus (10) and from the appearance of vaginal plugs. Organs were removed and cells were teased out by pressing between two glass slides. Single-cell suspensions were mixed with either BW5147 (11) or P.3U1 nonsecreting myeloma cells (12), pelleted, and fused with polyethylene glycol (PEG)[1] as previously described. Clones were expanded when they covered the bottom of microtiter plates. The frequency of

[1] *Abbreviations used in this paper:* FTL, fetal T cell hybrids from liver; FTT, fetal T cell hybrids from thymus; KLH-TNP, keyhole limpet hemocyanin-trinitrophenyl hapten.

hybrid cells was low and the success in long-term maintenance was 10% of the primary hybrids, largely due to an extensive crisis period of 5–6 wk after appearance of primary clones. Lines that survived this initial crisis showed vigorous in vitro growth over a 4–mo period.

Generation of +/nu^{str} and nu^{str}/nu^{str} lines. Bone marrow from two AKR nu^{str}/nu^{str} or two +/nu^{str} animals was removed under sterile conditions and fused with BW5147. Briefly described, 5×10^6 marrow cells were incubated with 5×10^6 BW5147 (AKR origin) hypoxanthine, aminopterin, thymidine (HAT)-sensitive thymoma cells in a V-bottomed tube. 0.5 ml of 40% PEG in RPMI (Kocklight Laboratories Ltd, Berkes, England) was added to pelleted cells for 1 min at room temperature. Cells were resuspended in serum-free RPMI by the dropwise addition of 15 ml over 10-min, centrifuged and washed once, resuspended, placed in a petri dish, and incubated overnight in complete media. Cells were then plated in HAT-selective media (18) and plated at limiting dilutions in microtiter wells. Growth-positive wells were expanded and tested. Equal numbers of wells (14/300 wells for both nu^{str}/nu^{str} and +/nu^{str}) showed growth. Cells were expanded and experienced 3- to 4-wk crisis periods before stable growth curves were achieved.

Monoclonal Antibody Specific for Tpre. Outbred nu/nu animals, originating from an attempt to breed nu/nu onto BALB/cAnN CrlBr (Charles River Breeding Laboratories, Inc., Wilmington, MA) were used. These animals differ from BALB/c at multiple loci but share the $Tthy^a$ allele at the $IgT-C$ region.[2] Male animals were immunized with C.AL-20 17-d fetal thymocytes (2×10^6) i.p. on day 14 and i.v. in phosphate-buffered saline on day 3. Cells were prepared in phosphate-buffered saline. Spleen cells were removed from the immunized mouse and fused with P.3U1 cells (12). Clones appeared on day 8, and tissue culture supernatant fluid was incubated with five ^{51}Cr-labeled fetal T cell hybrid lines, known to be negative for the Tthy, Tind, and Tsu antigens (4). Antibody and complement-mediated lysis was monitored as counts per minute of ^{51}Cr released.

Positive lines were then screened on 19-d fetal B cell hybrids. Tpre (F.6.9.1, aμK antibody-secreting line) was negative on 19-d B cell lines, but positive on several T cell hybrid lines. Line F.6.9.1 was selectively subcloned by limiting dilution in two sequential passages and frozen for later use. Data in this paper are based on the tissue culture supernatant from this line.

Antibody-Mediated Cytotoxicity. Antibody and selected rabbit complement were incubated with ^{51}Cr-labeled hybrid cell lines in a two-stage assay (13), or, alternatively, cells lysed were visually enumerated by eosin dye exclusion (4). Data are expressed in terms of a cytotoxic index (the number of cells killed by antibody minus those killed by complement divided by those killed by anti-Thy1.2 minus those killed by complement multiplied by 100). Arithmetic mean ± difference from the mean of duplicate determinations is shown.

Source of Animals. AKR nu^{str}/nu^{str} animals were obtained from The Jackson Laboratory, Bar Harbor, ME (14). "BALB/c nu/nu" and BALB/c AnNCrBr were purchased from Charles River Breeding Laboratories, Inc. C.AL-20, CB.20, and BAB/14 animals have been bred at Tufts University since 1978 from stock originating from M. Potter, National Institutes of Health. C.B.AL-1 and C.B.AL-4 were derived from breeding stock generously donated by R. Riblet, Center for Cancer Research, Philadelphia, PA.

Cortisone Treatment of Animals. The cortisone treatment of animals has been described (4).

Thymocyte Repopulation Studies. A report by Basch and Kadish (15) describes repopulation of mouse thymus in 760-rad-irradiated animals by syngeneic marrow, and we followed their protocol closely. Briefly described, C.AL-20 5-wk-old males were given 760 rad of whole-body irradiation from a ^{137}Cs source. 1 h after irradiation, each mouse was intravenously given 1.5 $\times 10^7$ bone marrow cells pooled from eight C.AL-20 age-matched males. These cells were pretreated with 1 μg of anti-Thy-1.2 monoclonal (IgM) antibody (16), 1 μg of anti-Tthy (IgM) (3), or 1 μg of anti-Tpre (IgM, F.6.9.1). Mice were killed at 10 d and thymocytes were counted. In the experiment we attempted to use complement of the recipient animal in situ. Alternatively, it is possible that removal in the spleen of aggregated cells could be the mechanism of depletion, since mouse complement is so inefficient in vitro. The survival rate of repopulated groups was in our open animal room. That the rate was not higher is possibly due to endemic viral pathogens known to exist in our colony.

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[2] Owen, F. L. Allelic expression of $Tthy^a$ and $Tthy^d$ in (BALB/c $\times$ C.AL-20)F$_1$. Manuscript in preparation.

The experiments were executed by pretreatment with antibody and not antibody and complement because our rabbit complement is very toxic to marrow cells in vitro. Mice (6) were treated intravenously in a separate experiment with 100 μl of either anti-Tpre or anti-Tthy tissue culture supernatant to ensure that the antibody alone would not deplete the thymus of a normal host. The mice used in the experiment were lethally irradiated with 760 rad gamma radiation, and control mice had few residual thymocytes. It seems unlikely that the sole target of the antibody is the recipient's thymocytes.

Results

Distribution of Products of the IgT-C Region in Fetal T Cell Hybrids. The Tthy^d alloantigen is not expressed on fetal T cell hybrids from 17-d thymus (FTT 17-1, FTT 17-2, FTT 17-3) (Table I), as previously reported (4). These lines were used to screen for a T cell antigen that might be expressed earlier in ontogeny than Tthy^d. The F.6.9.1 (anti-Tpre) line arose from a BALB/cAnNCriBR-related *nu/nu* animal (Charles River Breeding Laboratories, Inc.) that had been immunized with 17-d fetal thymocytes (see Materials and Methods). Spleen cells were removed 3 d after a third intravenous immunization with 2×10^5 cells in phosphate-buffered saline and fused with a nonsecreting variant of P.3U1. Tissue culture supernatant from developing clones was incubated with ^{51}Cr-labeled FTT 17-1, FTT 17-2, and FTT 17-3. Complement-mediated lysis was monitored by ^{51}Cr release. Positive tissue culture supernatants were then screened visually for lysis on each of the hybrid lines. Line F.6.9.1 produces μK antibody that reacted with many of the fetal T cell lines but failed to react with most of the control B cell hybrids (Table I) initiated from fusion of fetal liver from the same animals. One fetal liver T cell line, FTL 19-5, expressed all of the T cell antigens encoded by *IgT-C* (Tpre, Tthy, Tind, and Tsu). This unexpected observation is being pursued. Because lines FTT 17-1, FTT 17-3, and FTL 12-4 express Tpre^d (F.6.9.1) but not Tthy^d (17IIC6), one must conclude that Tthy^d and Tpre^d are on separate structural products.

Table II shows a panel of T cell hybrids constructed by fusion of adult lymph node cells with BW5147. Tpre and Tthy were expressed independently in these lines. Two lines expressing Tpre, SKTϕ220, and SKTϕ230, arose from peripheral cell fusion. Fig. 1 shows that high numbers of antigen-activated lymph node cells from the adult animal can absorb anti-Tpre. Therefore, it is not surprising that some cells from antigen-activated node (Table II) showed Tpre as a surface determinant. Lines 240 and 208 expressed Tthy but failed to express Tpre. One line (245) showed Tpre and Tthy coexpression. The frequency of adult lymph node hybrids expressing Tpre was <2% (data not shown). As many as 10% expressed Tthy. Table II shows only the lines that exhibited dual expression, not a complete panel of adult lines.

Tpre Is Expressed on Both Cortisone-sensitive and Cortisone-resistant Adult Thymocytes. Antibody specific for Tpre (F.6.9.1 hybridoma tissue culture supernatant) and complement lysed 25% of both cortisone-resistant and cortisone-sensitive thymocytes (Table III). The cell populations that expressed Tpre and Tthy may have partially overlapped, because the simultaneous addition of anti-Tpre and anti-Tthy did not increase the total number of cells lysed (Table IV).

Tpre is Encoded by the IgT-C Region of Chromosome 12. Strains of mice in which defined recombination events in and/or near the immunoglobulin loci on chromosome 12 occurred are shown in Table V. Thymocytes from each strain were evaluated on the same day to see whether they expressed surface antigens detectable by anti-Tpre

TABLE I

Expression of Tpre on Fetal T Cell Hybrids*

Fetal hybrid	Tpre‡	Tthy	Tind	Tsu	Thy-1.2	Lyt-1.2	Lyt-2.2
				T cell phenotype			
FTT 19-1	−	−	−	−	+	−	−
FTT 19-2	+	±	−	−	+	−	+
FTT 19-5	−	−	−	−	−	−	−
FTT 19-3	−	−	−	−	+	−	−
FTT 19-4	+	±	−	−	+	−	−
FTL 19-1	−	−	−	−	−	+	−
FTL 19-2	−	−	−	−	+	−	−
FTL 19-5	+	+	+	+	±	−	+
FTL 19-6	−	−	−	−	−	−	−
FTT 17-1	+	−	−	−	+	−	−
FTT 17-2	−	−	−	−	+	−	−
FTT 17-3	+	−	−	−	+	+	−
FTL 17-1	−	−	−	−	+	−	−
FTL 17-2	−	−	−	−	−	−	−
FTL 12-1	−	−	−	−	−	−	−
FTL 12-2	−	−	−	−	−	−	−
FTL 12-3	+	+	+	+	+	−	−
FTL 12-4	+	−	−	−	−	−	−
FTL 12-6	−	−	−	−	−	−	−
FBL 19-1	−	−	−	−	−	−	−
FBL 19-2	−	−	−	−	−	−	−
FBL 19-3	−	±	−	−	−	−	−
FBL 19-4	−	−	−	−	−	−	−
FBL 19-5	−	−	−	−	−	−	−
FBL 19-6	−	−	−	−	−	−	−
FBL 12-2	−	−	−	−	−	−	−
FBL 12-3	+	+	+	+	+	−	−
FBL 12-4	−	−	−	−	−	−	−

* Surface phenotype was established by visual observation of monoclonal antibody
and complement-mediated lysis and dye exclusion (17).

‡ Frequencies of positive cells in a given line varied from 10 to 30%.

(F.6.9.1) and complement-mediated lysis. Only the C.AL-20 strain expressed this antigen. The recombinations between known genes shown in Table V limit the genetic information coding for expression of Tpre to a short region of chromosome 12, previously called *IgT-C* (2). Because BALB/c (Igh-1^a) and BAB/14 (Igh-1^b) do not react with anti-Tpre^d, but presumably have this cell type, they must express a different allelic product. It is possible that there are three alleles for this antigen. Studies by Tokuhisa and Taniguchi (5, 6) suggest that BAB/14 may express a different allele for Tind or Tsu than does BALB/c or C.AL-20, which may be true for Tthy and Tpre as well.

Tpre is Expressed on Cells in the Bone Marrow of Adult Animals. The distribution

TABLE II

Expression of Tthy or Tpre on Adult T Cell Hybrids

Hybrid*	Tpre	Tthy	Tind	Tsu
SKO 220	+	−	−	−
SKO 230	+	−	−	−
SKO 245	+	+	+	−
SKO 240.6	−	+	+	−
SKO 240	−	+	+	−
SKO 208	−	+	+	−

* The cell lines shown in this panel were generated from fusion of adult lymph node from KLH-TNP footpad-challenged mice with BW5147 (S. K. Keesee, unpublished data).
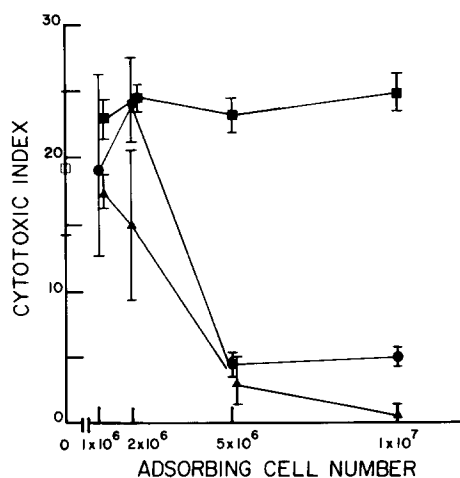

FIG. 1. Adsorption of anti-Tpre by H-2-primed lymph node cells, KLH-TNP-primed cells, or thymocytes from the C.AL-20 animal. 10^7 CAL.B spleen cells were injected into C.AL-20 mice i.p. on day 10, and 10^7 C.AL.B cells were injected into the footpad on day 4. Lymph node cells from the popliteal node (■) were used to adsorb 40 μl of tissue culture supernatant thymocytes. Alternatively, popliteal nodes from C.AL-20 mice challenged on day -28 and -4 in the footpads with 50 μg of KLH-TNP in CFA were used to adsorb antibody (●). C.AL-20 thymocytes (▲) were used as a positive control for adsorption.

TABLE III

Tpre Is Expressed on Both Cortisone-Sensitive and Cortisone-resistant Adult Thymocytes

Antibody	Comple-ment	Cortisone-treated*	Control
—	+	5 ± 1‡	4 ± 1
Anti-Tpre[d]	+	24 ± 6	30 ± 3
Anti-Thy-1.2	+	33 ± 1	86 ± 1

* As previously reported (17), ~20% of thymocytes in the C.AL-20 animal are resistant to 50 μg of cortisone phosphate at 48 h.

‡ Data are expressed as percent dead cells in duplicate determinations ± SE of the arithmetic mean. Antibody from tissue culture supernatant was incubated with cells, complement was added, and eosin red was added to aid in visualization of dead cells under phase-contrast microscopy.

TABLE IV

Adult Thymocytes Express Tthy^d and Tpre^d on Overlapping Populations

Antibody	Comple-ment	Cytotoxic index*
Anti-Tthy^d (20 μl) + anti-Tpre (20 μl)	+	16 ± 2
Anti-Tthy^d (20 μl, clone 17IIC6)	+	13 ± 1
Anti-Tthy^d (40 μl, clone 17IIC6)	+	13 ± 2
Anti-Tpre^d (20 μl, clone F.6.9.1)	+	15 ± 2
Anti-Tpre^d (40 μl, clone F.6.9.1)	+	12 ± 1
Anti-Tthy^d (20 μl, clone 17IIC6) and anti-Tthy^d (20 μl, clone 208IE6)	+	19 ± 1
Anti-Thy 1.2 (monoclonal)	+	69 ± 2

* Cytotoxic index is the number of dead cells in a given sample minus the number of dead cells in the complement control divided by the number of dead cells in the anti-theta control minus the dead cells in the complement control times 100. Complement killed 6% of the thymocytes.

TABLE V

*Tpre is Encoded by the IgT-C Region of Chromosome 12**

Strain	Cytotoxic index‡	Igh-V	Igh-C	IgT-C	H(Ig)	Pre
BALB/c	<1	a	a	a		0
C.AL-20	30 ± 5	d	d	d	d	a
BAB/14	<1	a	b	b	b	c
CB.AL-1	<1	d X	b	− X	d	a
C.B.AL-4	<1	d X	b	−	b	c

* Visual lysis of adult thymocytes by antibody and complement was used to type strains.
‡ See footnote, Table IV.

TABLE VI

Expression of Tpre on Adult C.AL-20 Bone Marrow

Antibody	Treatment of adsorbing cells	Comple-ment	Cytotoxic index*
T-pre	—	+	18 ± 3‡
T-pre	3 × 10^7 BM§ + C′‖	+	2 ± 4
T-pre	1 × 10^7 BM + C′	+	7 ± 2
T-pre	5 × 10^6 BM + C′	+	14 ± 1
T-pre	10^7 Thy-1.2 BM + C′	+	17 ± 3
T-pre	10^7 BAT¶ BM + C′	+	21 ± 5

* See footnote, Table IV.
‡ Antibody was used to lyse adult thymocytes from the C.AL-20 animal.
§ Bone marrow.
‖ Complement.
¶ Brain-associated T cell antigen.

pattern of Tpre on cells in the bone marrow was very much like that of Tthy (4) (Table VI). T cells sensitive to both rabbit anti-brain-associated T cell antigen (14) and anti-Thy-1.2 (16) bear Tpre. This was surprising inasmuch as the pre-T cell in marrow, which can repopulate the thymus of irradiated recipients, is reported to be Thy-1.2 surface negative (15). It seemed possible that many T cell precursors exist in

TABLE VII

Repopulation of Mouse Thymus in Irradiated Recipients with Adult Bone Marrow; Surface Phenotype of the Repopulating Cell

Group	Antibody* pretreatment of marrow	Cells transferred/recipient‡	Cells recovered at 10 d in recipient thymus§
1	None	1.5×10^7	$7.9 \times 10^6 \pm 4.6 \times 10^6$
2	None	None	2×10^4
3	Anti-Thy-1.2	1.5×10^7	$18 \times 10^6 \pm 6 \times 10^6$
4	Anti-Tthy^d	1.5×10^7	$9.2 \times 10^6 \pm 3.0 \times 10^6$
5	Anti-Tpre^d	1.5×10^7	$4.8 \times 10^4 \pm 2.8 \times 10^4$

* Syngeneic marrow was treated with 1 μg of monoclonal antibody per 1.5×10^7 cells for 30 min at 4°C. Cells in antibody were injected intravenously into recipients 1 h after irradiation with 760 rad ^{137}Cs. All antibodies are IgM K monoclonals.

‡ Cells were transferred intravenously.

§ Four animals were used. Data represent the arithmetic mean ± SEM of three separate surviving mice/group, not pooled cells from three mice. The experiment was terminated on day 10 due to mortality and morbidity in groups 2 and 5.

marrow and that the Tpre-bearing cell was not identical to that previously described (15). This was pursued experimentally (Table VII).

The data in Table VII show that a cell in C.AL-20 marrow, which expresses Tpre, is capable of repopulating the thymus of lethally irradiated recipients. Marrow from C.AL-20 animals was incubated with monoclonal anti-Tthy, anti-Tpre, or anti-Thy1.2. The normal marrow repopulated the thymus of syngeneic animals treated with 760 rad of gamma radiation, as did the anti-Thy-1.2- and anti-Tthy-treated groups. In contrast, pretreatment of marrow with anti-Tpre resulted in no repopulation (group 5) in contrast to the untreated irradiated animals (group 2). Group 5 showed evidence of morbidity. The survival rate in groups 1–5 was 75%; the untreated animals (75% in group 2) died within 10 d. Our results agree with those of Basch and Kadish (15), who show that the repopulating cell is Thy-1.2 surface negative.

Expresseion of Tpre in the AKR nu^{str}/nu^{str}. Limiting dilutions of cells from the AKR nu^{str}/nu^{str} (17) or AKR $+/nu^{str}$ age- and sex-matched animals were used to adsorb anti-Tpre. The residual antibody was then scored on thymocytes from a C.AL-20 adult male mouse. The AKR nu^{str}/nu^{str} bone marrow cells adsorbed at least 10 times less antibody than did bone marrow cells from the heterozygous $+/nu^{str}$ animal (Fig. 2). The patterns of adsorption by spleen cells from heterozygote and homozygote are almost identical. This experiment was performed three times. In two experiments, 10^6 marrow cells adsorbed 50% of the amount possible (data not shown). We did not perform additional experiments because large numbers of nudes were not available. Earlier studies had shown that nu^{str}/nu^{str} marrow cells do not express Tthy (4). The defect in nu^{str}/nu^{str} animals may prevent maturation of a common precursor to a daughter cell destined to express Tthy in $+/nu^{str}$ animals. The nature of the defect is unknown. Because the experiments summarized in Table VII suggest the Tpre$^+$ Tthy$^+$ cell in marrow may be missing in nude mice, the frequency of Tpre$^+$ cells was of critical interest. Attempts to estimate that frequency with antibody and complement failed because of complement backgrounds in marrow. The following alternative approach was taken.
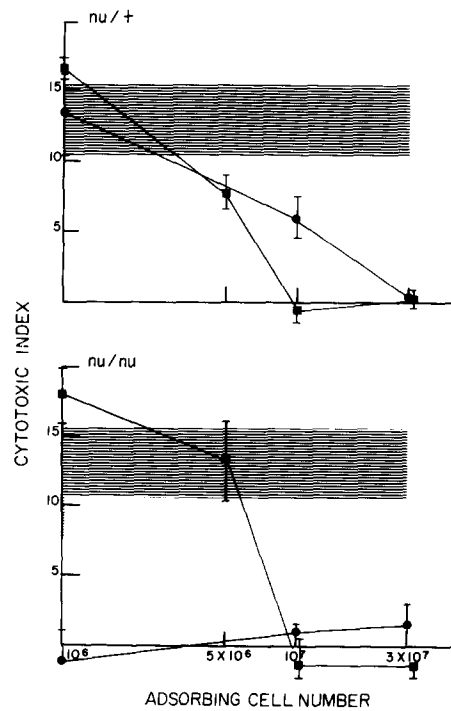
Fig. 2. Cells from AKR nu^{str}/nu^{str} bone marrow adsorb anti-Tpre. Limiting dilutions of marrow (●) or spleen cells (■) from nu^{str}/nu^{str} or $+/nu^{str}$ animals adsorb F.6.9.1. Residual antibody was scored on C.AL-20 adult thymocytes. Data are expressed on the basis of a cytotoxic index. The grey areas indicate the range of the cytotoxic index for unabsorbed antibody.

Distribution of Tpre, Tthy, Tind, and Tsu on Hybrids of nu^{str}/nu^{str} or $+/nu^{str}$ Bone Marrow Cells and BW5147. Hybrids of BW5147 (AKR) and AKR $+/nu^{str}/nu^{str}$ were grown. The number of growth-positive clones per marrow cell was identical for the two groups (14 clones per 5×10^6 cells), and one assumes that the fusions were comparable. These lines were typed with antibody and complement for surface expression of Tpre, Tthy, Tind, and Tsu. It is possible that AKR BW5147 may have a preferential fusion partner and that the state of differentiation of a given cell restricts its ability to stably fuse with the thymoma. Therefore, the lines developed may not be a random sampling of bone marrow. It is, however, apparent that the two groups of lines, which should be closely related, differ with respect to surface expression of Tpre (Table VIII). The only surface phenotype in the nude lines is Tpre$^+$, Tthy$^-$, in contrast to the $+/nu$ lines which show only Tpre$^+$, Tthy$^+$. Given that our sample size is small and that the number of lines that show any surface positive markers is smaller, the difference between the two groups is striking. This is consistent with a model in which the marrow cell in nude mice expresses Tpre and the Tpre$^+$, Tthy$^+$, Thy-1.2$^+$ cell that occurs in the heterozygote is either missing or very infrequent in the nude animal. The frequency of Tpre-positive cells in these lines is 50–75% for the cells from the nude mouse and 10–15% for the heterozygous lines. The density per cell of Tpre may be very different, explaining the greater efficiency of adsorption by marrow from nude animals shown in Fig. 2.

Distribution of Tpre on Antigen-Activated Lymph Node Cells. Tpre is expressed on

TABLE VIII

Expression of Tpre, Tthy, Tind, and Tsu* on Hybrids Originating from AKR
nu^{str}/nu^{str} or $+/nu^{str}$ Bone Marrow

	nu^{str}/nu^{str}					$+/nu^{str}$			
	Tpre	Tthy	Tind	Tsu		Tpre	Tthy	Tind	Tsu
A1	−	−	−	−	B6	+	+	−	−
A5	+	−	−	−	B4	−	−	−	−
A3	+	−	−	−	C1	+	+	−	−
B6	−	−	−	−	A2	+	+	−	−
B1	−	−	−	−					
B4	−	−	−	−	C2	−	+	−	−
C1	−	−	−	−	A1	+	+	−	−
B3	+	−	−	−	A5	+	+	−	−
A6	+	−	−	−	B2	−	−	−	−
B5	−	−	−	−	B3	−	−	−	−
B2	−	−	−	−					

* Surface phenotype of each line was determined by visual cytotoxicity using fluorescein isothiocyanate-modified monoclonal antibodies. Greater than 10% lysis above a complement background of 5% was considered significant. The lines from nu/nu animals were 30–50% positive for Tpre.

TABLE IX

Depletion of Tpre in Mice Neonatally Treated with Anti-Tthy

Mouse	Complement	Anti-Tthy-1.2	Anti-Tthy	Anti-Tpre
Control	8 ± 2*	48 ± 2	19 ± 1	20 ± 1
Experimental 1	7 ± 2	50 ± 5	9 ± 1	8 ± 1
Experimental 2	12 ± 1	43 ± 1	9 ± 1	7 ± 1

* Percent lysis of adult thymocytes.

TABLE X

Expression of Tpre on 17-d Fetal Thymocytes

	Anti-Tpre	Anti-Tthy	Anti-Tthy-1.2	Complement
D_{17} fetal cells	35 ± 5*	15 ± 4	43 ± 5	11 ± 3
Adult cells	28 ± 1	31 ± 5	59 ± 8	16 ± 4

* Percent lysis with antibody and complement ± SE of duplicate determinations. The Thy-1.2 control is unusually low in the adult. Two other experiments were completed with similar results but greater scattering of data points.

keyhole limpet hemocyanin-trinitrophenyl hapten (KLH-TNP)-activated lymph node cells with a frequency and/or density equal to that of the thymocyte pool (Fig. 2). In contrast, virgin lymph node cells and H-2^b-stimulated lymph node cells do not express Tpre in detectable amounts (Fig. 1). These studies were performed by quantitative adsorption. Tissue culture supernatant from F.6.9.1 was adsorbed with 1×10^6–3×10^7 cells from the popliteal lymph node of mice treated on days −10 and −4 with 50 μg of KLH-TNP in complete Freund's adjuvant. Alternatively, node cells of C.AL-20

(H-2^d) mice challenged with 10^7 spleen cells from C.AL.B (H-2^b) mice on days −10 and −4 also failed to adsorb anti-Tpre with a frequency higher than that of the resting cell population. Tpre is not detectable on the peripheral population by these assays.

Coexpression of Tthy and Tpre on Overlapping Populations in the Adult. We have developed a model for in vivo depletion of cells expressing Tthy.[3] Briefly described, neonatal mice are injected with anti-Tthy on days 0–1 and at 3-d intervals thereafter until they reach maturity. These animals fail to express cells having Tthy, Tind, or Tsu. When tested with antibody and complement, the Tpre-bearing cell is also depleted (Table IX).

Expression of Tpre on Fetal Thymocytes. Thymocytes were removed microscopically from 17-d fetal animals. Cells (1 × 10^5) were treated with anti-Tpre or anti-Tthy and complement. Anti-Tpre lyses a detectable number (25%) of fetal thymocytes, whereas anti-Tthy does not (Table X). Adult thymocytes express both Tpre and Tthy. Fetal liver cells do not express Tpre on day 13, 15, or 17 with a frequency/density detectable by direct lysis in this assay (data not shown). A more extensive fetal ontogeny study is in progress.

Discussion

A series of four T cell alloantigens, encoded within a short region of chromosome 12, have been described. Tpre, Tthy, Tind, and Tsu may be closely related products of a family of genes coding for antigenic determinants on regulatory T cells. These antigens are expressed sequentially in ontogeny and T cell maturation (Table IX) (18). The relationship of the cells expressing these alloantigens needs greater clarification. It has been shown that the cell that expresses Tthy is a probable common precursor for those cells expressing Tsu and Tind.[3] At present there is no evidence that Tpre is expressed on a precursor cell for the cell expressing Tthy, although this possibility is being explored. This cell does appear on or before day 17 in fetal thymus, before the appearance of cells expressing Tthy. Tpre and Tthy are expressed on largely overlapping cells in adult thymus (Tables IV and VIII). It therefore is not surprising that an occasional hybrid T cell arising from peripheral node expresses Tpre and Tthy simultaneously (Table II).

It is possible that these antigens represent a group of T cell-specific antigen receptors on cells that regulate immunoglobulin synthesis. If that is the case, a gene complex with an evolutionary link to the immunoglobulin genes might be expected to observe some of the same biological and organizational rules. One might expect an ordered sequence of genes on the chromosome, with characteristic genetic rearrangement mechanisms to conserve antigen-binding information; an adherence to the phenomenon of allelic exclusion of genes on the chromosome, with characteristic genetic rearrangement mechanisms to conserve antigen-binding information; and the existence of characteristic switch sites between gene segments. The immunoglobulin isotypes are distributed on B cells at characteristic points in B cell maturation. It is possible that T cell "isotypes" are similarly distributed and that Tpre, Tthy, Tind, and Tsu are the first four members of such a family.

The new alloantigen described in this paper, Tpre, is a separate antigenic specificity

[3] Keesee, S. K., and F. L. Owen. Neonatal modulation of Tthy. The cell bearing Tthy is required for the appearance of the cells expressing Tind and Tsu. *J. Exp. Med.* 157:86.

from that previously described as Tthy (3). Several lines of evidence establish this difference: (a) Tpre is expressed on three fetal T cell hybrids that do not express Tthy (Table I, lines FTT 17-1, FTT 17-3, and FTL 12-4) and two adult hybrids that do not express Tthy (Table II, SKO 220 and SKO 230). An occasional hybrid (Table II, SKO 240 and SKO 208) expresses Tthy and not Tpre. Many fetal and adult lines express these two antigens simultaneously. (b) Only the Tpre antigen is expressed on hematopoietic precursors for thymic repopulation (Table VII) or on a stem cell for the lymphoid lineage. Experiments to distinguish these possibilities are in progress. Anti-Tpre depletes cells from normal adult marrow capable of repopulation of the thymus in lethally irradiated adult mice. Anti-Tthy or anti-Thy-1.2 fail to do so. (c) Tpre is expressed in nude mice, whereas Tthy is not (Fig. 1) (18). (d) Tpre is expressed in fetal ontogeny before the expression of Tthy (Table IX). Despite these apparent differences in cell distribution during hematopoiesis, there is considerable overlap in the cellular distribution of Tpre and Tthy in the adult animal. Cortisone-sensitive and cortisone-resistant populations seem to express these antigens with equal frequency (Table III). Sequential or simultaneous addition of anti-Tpre or anti-Tthy to thymocytes does not result in additive lysis of cells (Table IV). Mice neonatally maintained with anti-Tthy fail to express Tthy[3] and also fail to express Tpre (Table IX). The distribution in adult bone marrow may also be partially overlapping. Treatment of bone marrow with anti-Tthy-1.2 and complement depletes cells that adsorb both anti-Tpre (Table VI) and anti-Tthy (17). However, pretreatment of marrow with anti-Tpre, Tthy, and Thy-1.2 shows only the cell that expresses Tpre independently of Thy-1.2, and Tthy is the hematopoietic precursor for thymic repopulation (Table VII). This implies that there are two cells in marrow expressing Tpre; one expresses Tpre and the other Tpre and Thy-1.2, possibly with Tthy. This is consistent with a model in which a Tpre-bearing cell arises before maturation of Tthy.

There is no direct evidence that Tpre is an antigen receptor on fetal cells (or any cell). The presence of such a receptor has been inferred from functional data showing that 19-d fetal liver contains precursor cells for alloreactive cytotoxic T lymphocytes (8). Cells that can be sensitized to alloantigen and allowed to mature in embryonic organ culture exist earlier in ontogeny (19). For these reasons, it has been suggested that the alloreceptor may be the most primitive of the family of T cell receptors (20). The ontological similarity in expression of Tpre and the receptor for alloantigen led to experiments examining expression of Tpre on alloreactive cells (Fig. 2). Adult lymph node cells sensitized to H-2[b] alloantigens do not express Tpre. A source of fetal T cells sensitized to alloantigen was not available for study. It is possible that cells in the alloreactive pathway undergo isotype switching in maturation and that adult cells use a different structure than fetal cells. There is no evidence at present for a relationship between Tpre and the antigen receptor on any alloreactive cells or fetal cells.

A potential relationship between the cell that expresses Tpre and the pre-T cell described by others (15, 19, 21, 22) needs further experimental clarification. The presence of this cell in the marrow of nude animals does suggest that it is a prethymic cell. In addition, the brief data in Table VII showing thymic repopulation capability make this a potential candidate for the elusive pre-T cell. At present, the functional capability of the cells in nudes expressing this antigen is being pursued. If the cell that

expresses Tpre is a direct precursor of those in the marrow that express Tthy and those in the periphery expressing Tsu and Tind, then an arrest in migration of Tpre-bearing cells from the marrow may be responsible for the absence of cells in nude expressing Tthy, Tind, or Tsu (18). It is possible that growth factors under thymic influence may be responsible for maturation of this lineage of cells from a single common precursor.

The expression of adult antigens on both "T" hybrids and "B" hybrids from fetal liver (12-d) was surprising (Table I). An ontogeny study (18) had shown that Tthy, Tind, and Tsu are expressed postnatally. The fusion partner for the hybrid cells is BW5147 (an AKR thymoma). It is possible that the adult thymoma cell has the genetic information ($Igh-1^d$) to complement the fetal cell and that the source of the adult antigens in the fetal hybrids is BW5147. BW5147 does not express any of these antigens on the surface or by [^{35}S]methionine labeling (unpublished observation). Genetic complementation could explain the precocious appearance of these antigens on the surface of fetal cells but not the fact that two fetal liver T cell lines express all four antigens simultaneously. In adult hybrids (18) no cells expressing all these antigens simultaneously appeared even though the same thymoma was used as the fusion partner. In addition, one line, FBL 12-3, expresses all these T cell antigens (and Thy1.2) but arose from P.3U1 (BALB/c chain myeloma) fusion to 12-d fetal liver. Transcomplementation is not a plausible mechanism for surface expression because the BALB/c animal has the Igh-1^a allotype; at the IgT-C region they are Tthy^a. This suggests there may be precursor cells in fetal liver that are not fully committed and differentiated. Only hybrids arising from fetal liver show this anomalous expression. This observation should be pursued at a molecular genetic level, and a careful fetal ontogeny study could be informative. Because Tthy, Tind, and Tsu are expressed postnatally, factors that influence maturation of functional T cells may also be required for the maturation of cells bearing Tthy, Tind, and Tsu from a common precursor. The bridge from fetal to adult development in normal mice seems to be blocked at a stage very much like that observed in the AKR nu^{str}/nu^{str} (Fig. 1), which lacks an immunocompetent thymus. Perhaps thymus-dependent polypeptides are required for sequential maturation of this lineage of cells. Growth factors, including T cell growth factor (23), may not be synthesized until postnatal development is complete. Although we have interpreted these data to imply that this series of four antigens represents antigen receptors, it is possible that they are differentiation antigens on regulatory cells and that similarities with the Ig products arise solely from an evolutionary relationship.

Summary

A new T cell alloantigen, Tpre, has been identified by monoclonal F.6.9.1 antibody. This antigen is encoded by a gene linked to the cluster of T cell antigens in the IgT-C region of chromosome 12 (Tthy, Tind, and Tsu). Tpre is distinct from Tthy, Tind, or Tsu because it is expressed on bone marrow cells of the AKR nu^{str}/nu^{str}, the thymus repopulating precursor cell in normal adult marrow, and normal fetal thymocytes. Several fetal and adult T cell hybrids express these antigens independently. Tpre and Tthy are expressed on largely overlapping cell populations in adult thymus.

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