

## MODULATION OF Tthy ALLOANTIGEN EXPRESSION IN THE NEONATAL MOUSE

### The Tthy-bearing Thymocyte Is a Precursor for the Peripheral Cells Expressing Tind and Tsu\*

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The differentiative pathway traversed by the B lymphocyte in response to an antigen is accompanied by the appearance of a number of a different constant region surface isotypes. Anti-isotype suppression studies have shown that neonatal suppression with anti- $\mu$  antibody leads not only to the failure of IgM-bearing cells to appear in the spleens of mice, but also to a failure of IgG bearing cells to develop (1-3). This may suggest a maturational pathway from pre-B to plasma cell in which the  $\mu$ -bearing B lymphocyte is the precursor cell for those cells bearing  $\delta$  and  $\gamma$  chains (3). The failure of  $\gamma_3$  to be normally expressed in the serum of immune CBA/N mice (4) suggests that the maturation sequence (5) may be regulated by complex biological mechanisms.

Three T cell alloantigens, Tthy<sup>d</sup>, Tind<sup>d</sup>, and Tsu<sup>d</sup>, have been described (6-10). The map position of the genes coding for these antigens, proximal to the Igh-1 locus on chromosome 12, has led to the hypothesis that these alloantigens represent multiple isotypes of the T cell receptor for antigen (9). Alternatively, it is possible that the region tentatively named IgT-C is a gene complex encoding T cell-specific differentiation antigens. Ontogenetic studies have indicated a maturational sequence of Tthy<sup>d</sup>, Tind<sup>d</sup>, Tsu<sup>d</sup> (10). The order of appearance of the alloantigens on immunocompetent cells, as well as the failure to demonstrate Tthy<sup>d</sup> on cells in the periphery, suggested the possibility that the cell bearing Tthy<sup>d</sup> may be the precursor for those bearing Tind<sup>d</sup> and Tsu<sup>d</sup>. The studies outlined here explore that possibility.

### Materials and Methods

*Mice.* C.AL-20 (H-2<sup>d</sup>, Igh-1<sup>d</sup>) allotype congenic mice on a BALB/cAnN (H-2<sup>d</sup>, Igh-1<sup>a</sup>) background were developed by Michael Potter (National Institutes of Health, Bethesda, MD) and have been bred within our animal facility at Tufts University since 1978.

*Treatment of Neonatal C.AL-20 Mice with Anti-Tthy<sup>d</sup>.* Monoclonal antibody recognizing Tthy<sup>d</sup> (17IIC6) (8) was purified from ascites fluid by (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitation, dialyzed, sterilized by Millipore filtration, and stored at -20°C. Mice were injected intraperitoneally with 10  $\mu$ g antibody within 24 h of birth. Injections were continued at 48-h intervals until the mice were killed between days 35 and 45, mimicking the protocol used to deplete  $\mu$ -bearing B cells (2, 11).

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Given a half-life of 5 d for mouse IgM, these mice should have circulating levels of anti-Tthy antibody at the time of bioassay.

*Antigen Priming of Lymph Nodes.* Keyhole limpet hemocyanin (KLH)<sup>1</sup> (Calbiochem, San Diego, Ca) was modified with picrylsulfonic acid (12). Antigen (50 µg in phosphate-buffered saline [PBS]) was emulsified in 50 µl Freund's adjuvant (Difco Laboratories, Detroit, MI) and injected into the footpads of adult animals on days 0 and -7. The draining popliteal lymph nodes were removed on day 10 and used as targets for antibody-mediated complement lysis (10).

*Amplification of Antibody-mediated Cell Lysis by Fluorescein Isothiocyanate (FITC)-modified Monoclonal Antibody and Mouse Anti-FITC Immunoglobulin.* Monoclonal antibody was collected from the ascites fluid of mice primed with pristane and inoculated with hybrids of P.3U1 and BALB/cAnN anti-C.AL-20 immune cells (6). Ammonium sulphate precipitated protein (10 mg) was modified by incubating with 0.5 M sodium carbonate (pH 9.5, containing 100 µg FITC) for 1 h at 4°C. Labeled protein was eluted from a G-25 Sephadex column in 0.1 M sodium phosphate. Antibody was stored at 4°C in 0.04 M NaN<sub>3</sub>. Limiting dilutions of FITC-modified antibody were incubated with thymocytes from either C.AL-20 or BALB/c animals for 30 min at 4°C. Affinity-purified mouse antibody specific for FITC was incubated with washed cells for 30 min at 4°C without NaN<sub>3</sub>, and pelleted cells were resuspended in selected rabbit complement. After incubation at 37°C for 45 min, cells were washed and stored at 4°C. Cell lysis was scored visually by eosin dye exclusion. FITC-modified anti-Tthy<sup>d</sup>, Tind<sup>d</sup>, and Tsu<sup>d</sup> were used at a concentration of 0.01 µg/10<sup>5</sup> cells. Data are expressed as cytotoxic index, calculated as follows: [(percent cells killed, experimental - percent cells killed, complement (C') alone)/(percent cells killed, anti-Thy-1.2 - percent cells killed, C' alone)] × 100. Base line studies describing the distribution of these antigens on cells in lymphoid organs of normal mice have been communicated (10).

*Adsorption.* Monoclonal antibody modified with FITC (0.2 µg) was incubated with limiting numbers (5 × 10<sup>5</sup>-2 × 10<sup>7</sup>) of lymphoid cells from normal or anti-Tthy<sup>d</sup> treated mice for 30 min at 4°C. Cells were then removed by centrifugation and the adsorbed antibody was evaluated in the standard cytotoxicity assay described above.

*Fluorescent Analysis of Cell Surface Antigens.* Cells prepared from spleen or thymus were suspended in PBS containing 2% horse serum and 0.04M NaN<sub>3</sub>. 1 × 10<sup>6</sup> cells were incubated with biotinylated monoclonal antibodies specific for Lyt-1.2, Lyt-2.2, and Thy-1.2 (Becton, Dickinson & Co., Sunnyvale, CA) or rabbit anti-mouse Ig (Fab')<sub>2</sub> fragments for 30 min at 4°C (6). Cells were washed and resuspended in fluoresceinated avidin (Vector Laboratories, Burlingame, CA) or goat anti-rabbit Ig (Meloy Labs, Springfield, VA), respectively, for 30 min at 4°C. Cells were then washed and scored for membrane fluorescence in a cytofluorograf (System 50H, Ortho Diagnostic Systems). This instrument is a single-cell measurement system using an argon laser beam excitation source, set at 488 nm and producing 1 W of light output. The fluorescence emitted by stained cells was collected and filtered with a 510-540 nm bandpass filter (Schott Optical Glass Inc., Duryea, PA). The signals produced by the photomultipliers in response to collected, focused fluorescence from each cell were integrated and represented as histograms, with the number of events plotted against increasing intensity of the signal. Data represent the spectrum and frequency of fluorescence of each population analyzed. The fluorescence histograms were gated by the light scattered at low angles to the incident beam, to exclude fluorescence from debris and dead cells.

## Results

*Tthy<sup>d</sup> Alloantigen Expression is Depressed in Neonatally Modulated Animals.* Previous studies (10) have shown that Tthy<sup>d</sup> appears in the neonatal thymus between 1 and 2 d after birth, although adult levels are not attained until approximately 21 d of age. Expression of Tthy<sup>d</sup> was monitored in adult animals that had received repeated injections beginning within 24 h of birth with a monoclonal antibody (17IIC6, µk)

<sup>1</sup> Abbreviations used in this paper: FITC, fluorescein isothiocyanate; KLH, keyhole limpet hemocyanin; PBS, phosphate-buffered saline; sIg, surface immunoglobulin.

TABLE I  
*Tthy<sup>d</sup> Alloantigen Expression in the Thymus of Anti-Tthy Neonatally  
 Modulated Mice\**

Animal number	Igh-1	Neonatal <sup>‡</sup> anti-Tthy	Cytotoxic index <sup>§</sup> ± SEM
Experiment 1			
C.AL-20 control	d	—	22 ± 3
BALB/c control	a	—	1 ± 0
C.AL-20 1-1 (Exp)	d	+	7 ± 1
C.AL-20 1-2 (Exp)	d	+	3 ± 2
C.AL-20 1-3 (Exp)	d	+	3 ± 2
C.AL-20 1-4 (Exp)	d	+	2 ± 1
Experiment 2			
C.AL-20 control	d	—	25 ± 3
BALB/c control	a	—	5 ± 2
C.AL-20 2-1 (Exp)	d	+	7 ± 1
C.AL-20 2-2 (Exp)	d	+	2 ± 1
C.AL-20 2-3 (Exp)	d	+	2 ± 1
C.AL-20 2-4 (Exp)	d	+	6 ± 2

\* Data represent eight experimental animals from three separate litters. Experiments were performed on two days. Assay was antibody-mediated cytotoxicity as described in Materials and Methods.

<sup>‡</sup> Mice were treated with a monoclonal antibody recognizing Tthy<sup>d</sup> as described in Materials and Methods.

<sup>§</sup> Cytotoxic index = [(percent dead, anti-Tthy<sup>d</sup> – percent dead, C' alone) / (percent dead, anti-Thy-1.2 – percent dead, C' alone)] × 100. Data represent the average of duplicate samples.

specific for this antigen. The percentage of Tthy<sup>d</sup>-bearing cells in the thymus of neonatally treated and normal age-matched control animals was assessed by a cytotoxicity assay using FITC-modified Tthy<sup>d</sup>-specific monoclonal antibodies (17IIC6) plus affinity purified mouse anti-FITC antibodies and complement (Table I). The frequency of Tthy<sup>d</sup> positive cells was markedly decreased in treated animals, representing a mean cytotoxic index of 4 compared with 23 in control thymocyte populations. This observation suggests that treatment of newborn animals with antibodies specific for Tthy<sup>d</sup> results in the elimination of a substantial portion of the Tthy<sup>d</sup> bearing thymocyte population. Alternatively, anti-Tthy<sup>d</sup> antibody could act in vivo at the level of the antigenic determinant itself, resulting in capping and clearance of the cell membrane without lysing the cell bearing that determinant. In either case, we would detect fewer numbers of Tthy<sup>d</sup> positive thymocytes. Although our results do not distinguish between the two possibilities, the monoclonal antibody used for the in vivo modulation of Tthy<sup>d</sup> (17IIC6) is an IgM antibody, and therefore it is likely that its attachment to the surface of Tthy<sup>d</sup>-bearing cells would result in complement-mediated lysis of the cell and not simple antigenic blockade.

Newborn animals treated with anti-Tthy<sup>d</sup> but not its allelic counterpart, anti-Tthy<sup>a</sup>,<sup>2</sup> showed decreased levels of Tthy antigen expression (Table II). Because both of these monoclonal antibodies share the same isotope ( $\mu$ ,  $\kappa$ ), Tthy loss is probably not due to stress factors resulting from the immunization protocol. Both anti-Tthy<sup>a</sup> and

<sup>2</sup> F. L. Owen. Products of the IgT-C region of chromosome 12. Allelic expression of Tthy<sup>a</sup> and Tthy<sup>d</sup> in the (BALB/c × C.AL-20)F<sub>1</sub> heterozygote. Manuscript in preparation.

TABLE II  
*Neonatal Pretreatment with Anti-Thy<sup>a</sup> or Anti-Thy<sup>d</sup>*

Group number*	Antibody treatment‡	Cytotoxic index§ ± SEM
1	Anti-Thy <sup>a</sup>	14 ± 2 (5)
2	Anti-Thy <sup>d</sup>	2 ± 1 (3)
3	Control	18 ± 1 (5)

\* Groups represent experimental animals from separate litters. Assay was antibody-mediated cytotoxicity as described in Materials and Methods.

‡ Mice were treated with monoclonal antibodies recognizing Thy<sup>a</sup> (C.B.13.10) or Thy<sup>d</sup> (17IIC6) as described in Materials and Methods. Anti-Thy<sup>a</sup> (C.B.13.10) is described in Results.

§ Cytotoxic index = [(percent dead, anti-Thy<sup>d</sup> - percent dead, C' alone) / (percent dead, anti-Thy1.2 - percent dead, C' alone)] × 100. Data represent the average of duplicate samples. Numbers in parentheses represent number of mice per group. Each animal was evaluated individually and data represent the mean of five separate animals.

TABLE III  
*Flow Cytometer Analysis of Lyt-1.2, Lyt-2.2, Thy-1.2, and sIg Expression In Anti-Thy<sup>d</sup> Neonatally Modulated Mice\**

	Animal number	sIg	Number of positive cells ± SEM§		
			Thy-1.2	Lyt-1.2	Lyt-2.2
Experiment 1	Control	0.9 ± 0	98 ± 0	95 ± 2	78 ± 1
Thymus	1.1	5 ± 1	98 ± 0	95 ± 4	73 ± 8
	1.2	4 ± 1	94 ± 1	95 ± 1	59‡ ± 6
	1.3	1 ± 0	99 ± 0	95 ± 0	80 ± 1
	1.4	2 ± 0	98 ± 0	95 ± 0	76 ± 1
	Control	1 ± 0	95 ± 1	95 ± 1	64 ± 3
Experiment 2	Thymus	2.1	98 ± 0	95 ± 1	75 ± 2
	2.2	0.9 ± 0	97 ± 0	95 ± 3	56 ± 2
	2.3	0.4 ± 0	97 ± 0	95 ± 3	65 ± 0
	2.4	0.9 ± 0	97 ± 0	95 ± 1	58 ± 2
	Control	33 ± 2	43 ± 1	38 ± 1	20 ± 0
Experiment 1	Spleen	1.1	53 ± 0	34 ± 2	31 ± 1
	1.2	52 ± 2	38 ± 1	31 ± 1	18 ± 2
	1.3	47 ± 1	43 ± 0	38 ± 0	18 ± 1
	1.4	40 ± 7	43 ± 0	43 ± 1	21 ± 0
Experiment 2	Spleen	Control	32 ± 1	36 ± 1	19 ± 0
	2.1	43 ± 3	38 ± 1	36‡ ± 1	19 ± 2
	2.2	28 ± 1	39 ± 1	28 ± 14	11‡ ± 1
	2.3	31 ± 3	36 ± 0	21 ± 0	14 ± 0
	2.4	31 ± 1	39 ± 1	20 ± 0	18 ± 3

\* Data represent eight experimental animals from three separate litters. Experiments were performed on different days. Data were collected on an Ortho Diagnostics Cytofluorograf Flow Cytometer as described in Materials and Methods.

‡ No significant differences were observed in fluorescence patterns of neonatally modulated animals as compared with normal controls. These three animals represent unexplained individual variations.

§ Percent positive cells was determined as described in Materials and Methods from duplicate samples. Nonspecific staining for FITC-avidin and FITC-rabbit anti-mouse Ig were as follows: FITC avidin, spleen, 5.8 ± 2%, thymus, 1.9 ± 0.5%, FITC-RAM1g, spleen, 0.8 ± 0.8%, thymus, 0.4 ± 0.2%. Standard errors are rounded to the nearest whole number.

anti-Tthy<sup>d</sup> were ammonium sulphate-precipitated proteins raised in the ascites from pristane primed mice injected with P.3U1-derived hybrid cells.

*Modulation of Tthy<sup>d</sup> Does Not Affect Expression of Lyt-1.2, Lyt-2.2, Thy-1.2, or Surface Ig (sIg).* Surface expression of the T cell antigens Lyt-1.2, Lyt-2.2, Thy-1.2, and a marker for B cells, surface membrane immunoglobulin, was studied by flow cytometer analysis of the spleen and thymus cells of neonatally modulated animals. The results of this study (Table III) indicated no significant differences between anti-Tthy<sup>d</sup>-treated or control animals in the percentages of cells bearing any of these antigens. We would conclude that the cell bearing Tthy<sup>d</sup> represents a subpopulation of cells in the thymus, and that it does not have a direct role in regulating the differentiation of those cells characterized by various Ly phenotypes. The fact that we did not see a decrease in the total number of T cells by the criteria of cell sorter analysis (Thy 1.2) or direct counts (an average of  $8.3 \times 10^7$  vs.  $7.4 \times 10^7$  cells/thymus, anti-Tthy<sup>d</sup> treated, or control, respectively) in the thymuses of treated animals which had demonstrated a loss of Tthy<sup>d</sup> expression suggests the possibility that homeostatic mechanisms existing in vivo resulted in a compensatory increase in a different T cell subpopulation in these animals. This phenomenon has been observed for B cells in both allotype and idotype suppression studies (13, 14).

An examination of the histograms generated by flow cytometer analysis (Figs. 1-3) revealed that surface staining characteristics of experimental and control animals were virtually identical for all the antigens studied. This suggested that not only the number of cells but also the surface determinant density was unaltered by Tthy<sup>d</sup> antigenic modulation. sIg staining of thymus and spleen was not elevated. It is thus unlikely that IgM antibodies (anti-Tthy<sup>d</sup>) circulate and block Tthy<sup>d</sup> in vitro.

*Tind<sup>d</sup> and Tsu<sup>d</sup> Alloantigen Expression Is Altered in Neonatally Modulated Animals.* Studies by Owen (10) have indicated that the T cell alloantigens Tind<sup>d</sup> and Tsu<sup>d</sup> appear sequentially in peripheral lymphoid organs of neonatal mice, on days 2-3 and 5-6, respectively. Tthy<sup>d</sup> is expressed in the bone marrow and thymus, and its early appearance (days 1-2) suggested that the cell bearing this antigen may be a precursor for the cells expressing Tind<sup>d</sup> and Tsu<sup>d</sup>. Expression of Tind<sup>d</sup> and Tsu<sup>d</sup> was monitored in neonatally treated mice by indirect adsorption of monoclonal antibodies specific for these antigens. Spleen cells from mice that had been treated with anti-Tthy<sup>d</sup> were used to adsorb FITC-modified monoclonal antibodies specific for Tind<sup>d</sup> and Tsu<sup>d</sup>. The adsorbed antibodies were then evaluated in the standard complement-mediated cytotoxicity assay, using trinitrophenyl-KLH-primed lymph node cells as targets (Fig. 4). Tthy<sup>d</sup> expression was similarly measured by adsorbing the appropriate antibody with increasing numbers of thymocytes and then scoring the adsorbed antibody on thymus cells. Tind<sup>d</sup> and Tsu<sup>d</sup> expression was substantially depressed in anti-Tthy<sup>d</sup> neonatally modulated animals. An examination of panels A and B of Fig. 4 shows that the number of cells from modulated animals required to remove antibody activity was at least 10-fold greater than that of age-matched controls. These data provide additional evidence that treatment of neonatal mice with anti-Tthy<sup>d</sup> results in decreased numbers of Tthy-bearing cells, rather than modulation or masking of determinants without eliminating cells expressing Tthy. We have established in earlier experiments (8) that Tthy, Tind, and Tsu do not co-modulate at the cell surface. The monoclonal antibodies do not compete for the same site(s), suggesting that antigeni-

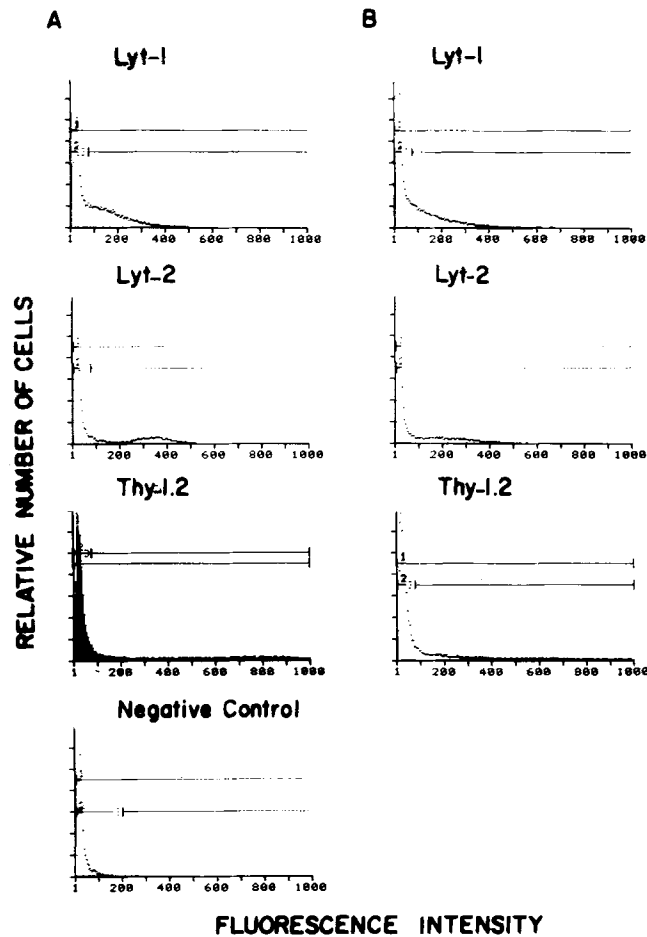


FIG. 1. Fluorescence analysis of spleen cells from anti-Tthy<sup>d</sup>-treated and control mice. Panel A, anti-Tthy<sup>d</sup>-treated animals; panel B, control animals.

cally unrelated determinants are depleted. Our results suggest that the cell bearing Tthy<sup>d</sup> is required for the later maturation of cells expressing Tind<sup>d</sup> and Tsu<sup>d</sup>.

### Discussion

In the studies reported here, we have used an immunization protocol analogous to that used to achieve chronic  $\mu$  suppression in neonatal mice with the goal of removing T lymphocytes bearing the Tthy<sup>d</sup> alloantigen. Tthy<sup>d</sup> is found on a subpopulation of thymocytes in (Igh-1<sup>d,e</sup>) mice but is not expressed at detectable levels on peripheral T cells (8, 10). This alloantigen is one of a group of allotype-specific T cell surface antigens reported in previous work from this laboratory. Recently, it has been demonstrated that Tthy<sup>d</sup> alloantigen expression precedes that of two other T cell alloantigens, Tind<sup>d</sup> and Tsu<sup>d</sup>, in ontogeny (10). This result, and the fact that Tthy<sup>d</sup> expression precedes that of Tind<sup>d</sup> or Tsu<sup>d</sup> on immunocompetent T cells, led us to investigate a possible precursorial relationship between the cell bearing Tthy<sup>d</sup> and

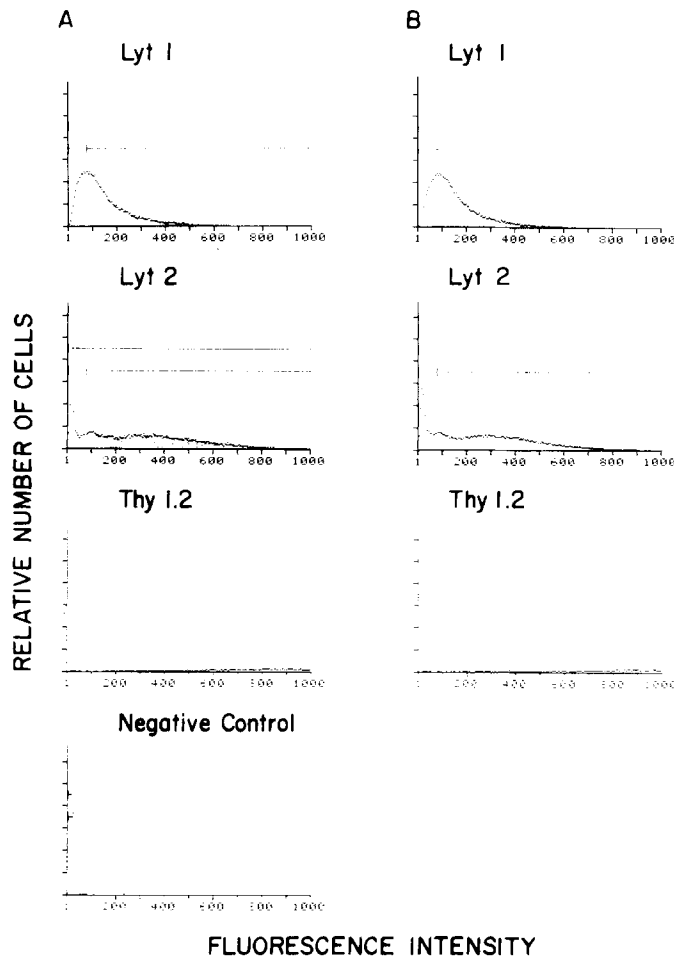


FIG. 2. Fluorescence analysis of thymocytes from anti-Tthy<sup>d</sup>-treated and control mice. Panel A, anti-Tthy<sup>d</sup>-treated animals; panel B, control animals.

those expressing Tind<sup>d</sup> and Tsu<sup>d</sup>. Treatment of newborn mice with a monoclonal antibody recognizing Tthy<sup>d</sup> resulted either in the complete abrogation or the severe depletion of thymocytes bearing this alloantigen (Table I). Tthy<sup>d</sup> expression has been monitored in >30 suppressed mice and in each case there has been significant depletion or the complete removal of Tthy<sup>d</sup>-bearing cells. These results are consistent with, but do not prove, the conclusion that treatment of mice from birth with monoclonal anti-Tthy<sup>d</sup> antibody results in the removal of the thymocyte subpopulation expressing this alloantigen. The apparent loss of the Tthy<sup>d</sup>-bearing T lymphocyte in neonatally suppressed animals could have a trivial explanation in that the antibody recognizing Tthy<sup>d</sup> (17IIC6) could block or mask the antigenic sites in such a way that they are not detected by our cytotoxicity assay. Such blocking could occur directly, or modulation at the cell surface could result in capping and endocytosis of the Tthy<sup>d</sup> antigenic determinant. However, the fact that Tind<sup>d</sup>- and Tsu<sup>d</sup>-bearing cells fail to appear in the peripheral lymphoid organs of suppressed animals (Fig. 4) argues

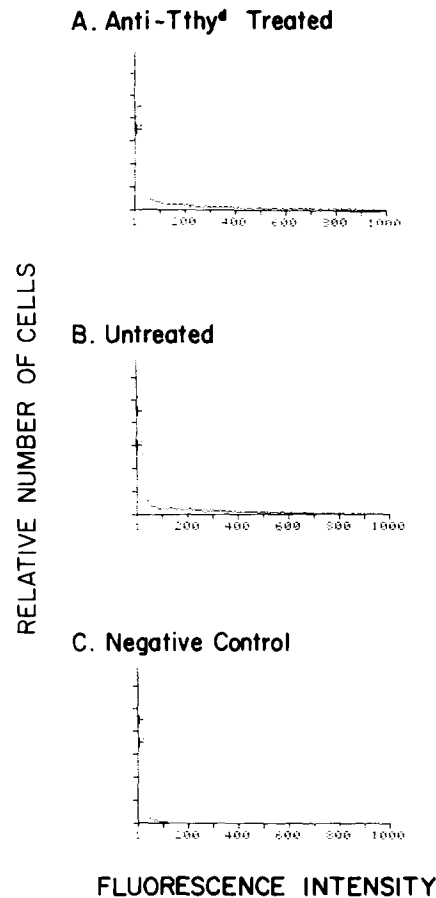


FIG. 3. sIg fluorescence analysis of spleen cells from anti-Tthy<sup>d</sup>-treated and control mice.

strongly against this possibility. It has been previously shown that the monoclonal antibodies recognizing Tthy<sup>d</sup>, Tind<sup>d</sup>, and Tsu<sup>d</sup> are non-cross-reacting (8).<sup>3</sup> Furthermore, Tthy<sup>d</sup>, Tind<sup>d</sup>, and Tsu<sup>d</sup> are expressed on largely nonoverlapping T lymphocyte subpopulations (10).

Cytofluorograph analysis of spleen and thymus cells from suppressed mice failed to show any alteration in levels of Lyt alloantigen or Thy-1.2 antigen expression, indicating that the pathway including the Tthy<sup>d</sup>-bearing cell is phenotypically distinct from that involving the development of various Ly phenotypes. Experiments performed on suppressed mice at 5, 10, or 15 d of age similarly failed to show any alteration of Lyt-1.2, Thy-1.2, or sIg expression (data not shown), indicating that these two pathways develop independently from an early age. If *in vivo* compensation between T cell subsets is obscuring a shift in Ly or Thy-1.2 antigen expression, then it must occur before the age of 5 d postpartum. The chromosomal location of the genes encoding the products of the IgT-C locus (chromosome 12) (9) is distinct from those responsible for Lyt-1.2 (chromosome 19) (15) or Lyt-2.2 (chromosome 6) (16).

<sup>3</sup> G. M. Spurrill, M. Frye, L. Riendeau, A. Finnegan, and F. L. Owen. A panel of monoclonal antibodies specific for products of IgT-C. Manuscript in preparation.



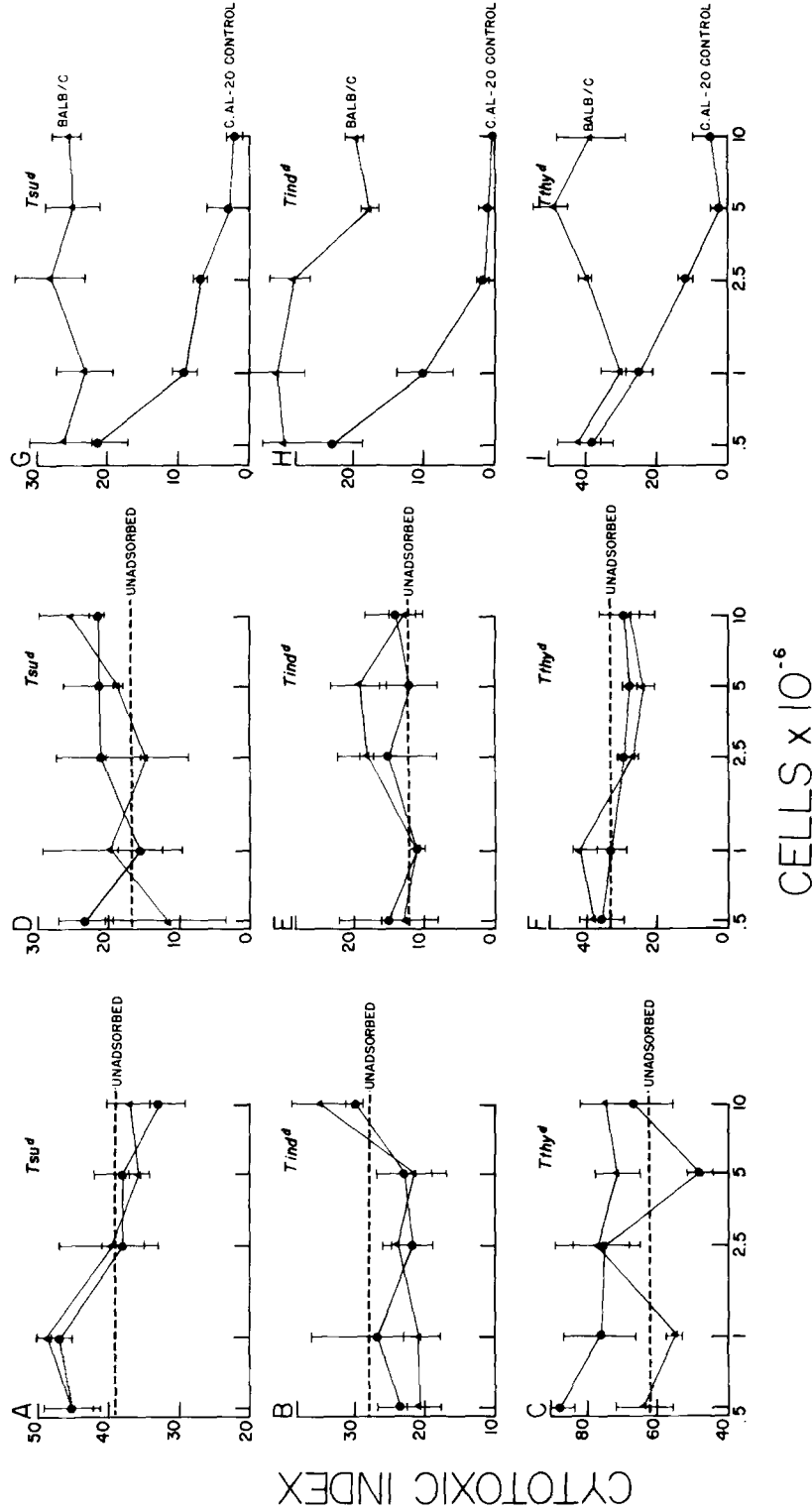


FIG. 4. Spleen cells (panels A, B, D, E, G, H) and thymocytes (panels C, F, I) were used to adsorb antibodies recognizing Tthy<sup>d</sup>, Tind<sup>d</sup> and Tsu<sup>d</sup>. Adsorbed antibody was evaluated in the standard cytotoxicity assay using antigen-primed lymph node cells (anti-Tind<sup>d</sup>, Tsu<sup>d</sup>) or thymocytes (anti-Tthy<sup>d</sup>). Panels A-F, anti-Tthy<sup>d</sup> neonatally modulated animals; panels G-I, control animals. C.AL-20 (Igh-1<sup>b</sup>), ●, BALB/CAnN (Igh-1<sup>a</sup>), ▲. Dashed line in each panel represents the level of target cell killing by unadsorbed antibody.

Lyt-1.2 is expressed on virtually all T lymphocytes (19). These findings suggest that the family of T cell alloantigens including Tthy<sup>d</sup> represents a group of cell surface markers delineating aspects of T cell development independent from those characterized by Ly phenotype (18).

The results of this study are consistent with the hypothesis that the T cell alloantigens Tthy<sup>d</sup>, Tind<sup>d</sup>, and Tsu<sup>d</sup> characterize a T cell differentiation pathway in which the Tthy<sup>d</sup>-bearing cell is a precursor for those expressing Tind<sup>d</sup> and Tsu<sup>d</sup>. Alternatively, Tthy<sup>d</sup> could be a surface marker for a regulatory cell that governs the differentiation of a separate precursor into cells expressing Tind<sup>d</sup> and Tsu<sup>d</sup>. We suggest that these three alloantigens may represent part of a group of T cell constant region isotypes, or possibly a collection of immunoglobulin gene complex-associated differentiation antigens, which serve as cell surface markers for distinct stages in T cell development. Although our results do not distinguish between these possibilities, it is worthwhile to note the parallel between these studies and those involving B lymphocyte differentiation in the mouse. Kearney et al. (19) have shown that B lymphocytes bearing sIgM precede those expressing sIgG. Studies involving suppression in neonatal mice have demonstrated that removal of sIgM<sup>+</sup> B lymphocytes also prevents the normal development of cells bearing any other surface immunoglobulin heavy chain isotype (1), whereas treatment of neonates with anti- $\delta$  antibodies results in a population of B cells that are phenotypically sIgM<sup>+</sup> sIgD<sup>-</sup> (20). These and recent similar findings of Fultz et al.<sup>4</sup> have led to the hypothesis that the ordered appearance of the surface immunoglobulin isotypes characterize distinct developmental stages in B cell differentiation (21).

The availability of a group of cell surface markers for distinct subsets of T lymphocytes provides one of the opportunities to examine in detail questions concerning the functional role of different T cell subpopulations in various immune phenomena. Ceredig et al. (22) have described an immunologically mature, Lyt 2<sup>+</sup> thymus cell subpopulation that produces interleukin-2. Both the cell size of this subset (medium) and its distribution in the thymus (15%) are consistent with the possibility that this population of cells represents those expressing Tthy<sup>d</sup>. It would be of interest to determine whether anti-Tthy<sup>d</sup> neonatally suppressed animals are capable of producing normal levels of interleukin-2.

Chronic suppression studies involving B lymphocytes have been useful in discerning possible functional interrelationships which exist between the B and T cell compartments of the immune response. Thus, Bottomly et al. (11) have shown that treatment of newborn mice with anti- $\mu$  antibody leads to the disappearance of not only sIg<sup>+</sup> B lymphocytes but also an antigen-specific T helper cell required for the expression of the T15 idiotype. Similarly, studies by Fultz and co-workers (23) have indicated that chronic suppression of an IA<sup>k</sup> specificity results in the depletion of surface IgM<sup>+</sup> B lymphocytes as well as significantly decreased MLR responses. We have demonstrated an in vivo model involving chronic T cell suppression. This model may be useful in the elucidation of the functional role(s) of various T cell subsets.

### Summary

The T cell alloantigen Tthy<sup>d</sup> appears on a subpopulation of thymocytes in mice bearing the Igh-1<sup>d</sup> or <sup>e</sup> heavy chain haplotype. Ontogenetic studies have suggested

<sup>4</sup> M. J. Fultz, I. Scher, F. D. Finkelman, P. Kincade, and J. J. Mond. 1982. Neonatal suppression with anti-Ia antibody. I. Suppression of murine B lymphocyte development. *J. Immunol.* 129:992.

that this antigen precedes the appearance of two other T cell alloantigens in the same linkage group, Tind<sup>d</sup> and Tsu<sup>d</sup>. The purpose of this study was to determine if the Tthy-bearing cell is a precursor for cells in the periphery expressing Tind and Tsu. C.AL-20 mice were treated at 48-h intervals beginning on the day of birth with a monoclonal antibody recognizing Tthy. Tthy alloantigen expression, monitored by cytotoxicity assays, was found to be significantly depressed in the thymuses of treated animals; Tind and Tsu also failed to appear in the periphery. Treatment with anti-Tthy caused no significant changes in frequency or surface intensity in the expression of surface Ig, Thy-1.2, Lyt-1.2, and Lyt-2.2, as studied by cytofluorograph analysis. We conclude that the T-thy<sup>d</sup>-bearing cells in the thymus represent a subpopulation that may be a precursor for Tind<sup>d</sup>- and Tsu<sup>d</sup>-bearing cells. However, Tthy<sup>d</sup>-bearing cells are more mature than the Thy-1.2 common T cell precursor, pre-T.

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