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Immunology

Monoclonal antibodies specific to native murine T-cell receptor γδ: Analysis of γδ T cells during thymic ontogeny and in peripheral lymphoid organs

(CD4 and CD8 molecules/T-cell receptor αβ/mouse)

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ABSTRACT **Three hamster monoclonal antibodies (mAbs), all recognizing different epitopes present on the native form of the murine T-cell antigen receptor (TCR) γδ subunits, have been generated. mAb 3A10 is specific to a pan-murine TCR γδ, recognizing a C_δ constant region determinant. mAb 8D6 is specific to a subset of T cells expressing $V_{\gamma 4}$- and $V_{\delta 5}$-encoded γδ TCR, and mAb 5C10 is clonotypic. Using these and other mAbs directed against a variety of T-cell surface markers, we quantitated and characterized γδ T cells present in developing thymuses as well as in the conventional lymphatic organs by flow cytometry. These studies revealed that (*i*) many γδ thymocytes and peripheral T cells bear CD4 and/or CD8 molecules, (*ii*) T cells bearing both αβ and γδ TCRs are scarce, and (*iii*) thymocyte subsets bearing TCR γδ encoded by different combinations of V_γ and V_δ gene segments appear in waves during ontogeny.**

One of the most critical steps in the vertebrate immune response is the recognition of antigens by lymphocytes. This task is accomplished by two sets of glycoproteins, immunoglobulins and T-cell antigen receptors (TCRs). The most extraordinary feature of these proteins is their structural variability, much of which originates from the ability of the encoding gene segments to undergo somatic rearrangement (1). All TCRs were initially thought to be composed of a heterodimeric protein composed of α and β subunits. However, the search for the genes encoding these polypeptides led to the identification of a third rearranging gene (2, 3), which was later shown to code for one of the two subunits of another heterodimer, TCR γδ (4, 5).

Despite the striking similarities in the overall structure of their genes and polypeptide chains, TCR γδ and the T cells which express it are significantly different from their αβ counterparts. For instance, γδ T cells are detected in both the thymus (6–10) and peripheral lymphoid organs (5, 11, 12) in relatively low quantity (<5% of T cells). However, γδ T cells predominate (50–100%) within epithilia, such as epidermis (13, 14) and small intestine (15, 16). Furthermore, the majority of γδ T cells in the thymus and spleen do not express either CD4 or CD8 molecules (5–12, 17), which are found on most αβ T cells. Finally, neither the specificity of γδ TCR recognition nor the function of γδ T cells in immune defense is understood.

To determine the nature of the TCR γδ and the role of γδ T cells, monoclonal antibodies (mAbs) directed against the native receptor would be extremely useful. This report describes the isolation of three different TCR γδ mAbs, each having its own specificity for distinct γδ T cell populations. Initial characterization of mouse thymocytes and peripheral T cells by using these mAbs and others directed at various T-cell surface markers revealed several new aspects of γδ T cells.

MATERIALS AND METHODS

Animals and Cells. Armenian hamsters and all strains of mice were from Cambridge Diagnostic (Cambridge, MA) and The Jackson Laboratory, respectively. Thymocytes and peripheral lymphocytes were purified by density gradient centrifugation using Lympholyte M (Cedarland Laboratories, Hornby, ON, Canada) and by the panning method using affinity-purified goat anti-murine IgG and IgM antibodies (Kirkegaard and Perry Laboratories, Gaithersburg, MD). T-cell hybridomas expressing TCR γδ have been described (18, 19). Murine myeloma SP2/0 was used as the B-cell fusion partner.

Antibodies and Reagents. The mAb 145-2C11 (anti-CD3) (20) was used as culture supernatant and a fluorescein isothiocyanate (FITC) conjugate. The mouse mAb KN365 recognizing denatured TCR γ chains has been described (11, 16). The hamster mAb 597 (anti-TCR β) (21) was used as culture supernatant and a biotin conjugate. Phycoerythrin (PE) conjugate of mAb GK1.5 (anti-CD4) and biotin and FITC conjugates of mAb 53-6.7 (anti-CD8) were from Becton Dickinson. Goat anti-hamster IgG FITC conjugates were from Kirkegaard and Perry Laboratories and Caltag Laboratories (San Francisco). The FITC conjugate of mAb MAR-18 (mouse anti-κ chain of rat immunoglobulin) was from Thereza Imanishi-Kari (Tufts University, Boston). Streptavidin-PE and streptavidin-DuoCHROME conjugates were from Becton Dickinson.

Immunization and Fusion Protocols. For immunization, anti-CD3 immunoprecipitates (protein A-Sepharose/anti-CD3/CD3-γδ complex) were prepared from a 1% digitonin-solubilized lysate of a γδ T-cell hybridoma, KN6 (19). The immunoprecipitates were injected intraperitonealy into the hamsters four times with 3- to 6-week intervals between injections. We used Freund's complete and incomplete adjuvants for the first and subsequent immunizations, respectively. The spleens were removed 3 days after the last injection. The spleen cells were fused to SP2/0 myeloma cells

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Abbreviations: TCR, T-cell antigen receptor; mAb, monoclonal antibody; FITC, fluorescein isothiocyanate; PE, phycoerythrin; V, variable; C, constant; E *n*, embryonic day *n*; DN, double-negative; SP, single-positive; DP, double-positive.

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and cultured according to the procedure described by Lane (22).

Cell Surface Radiolabeling and Immunoprecipitation. Cell surface radioiodination and immunoprecipitation of the reduced or nonreduced lysates were performed as described (9, 11).

Flow Cytometric Analysis and Fluorescense-Activated Cell Sorting. Single-cell suspensions were stained by the standard procedures. The samples were analyzed with a single-beam flow cytometer, FACScan (Becton Dickinson). In the case of single- or double-color analysis, we used propidium iodide (0.5 μg/ml) to eliminate the dead cells from the data. In the case of triple-color analysis, we used highly viable cell populations (at least 96%) for the staining and carefully gated live cells by forward and side light scattering. The data were analyzed with the FACSCAN Research Software (Becton Dickinson). Cell sorting was carried out with Epics C (Coulter).

RESULTS AND DISCUSSION

Generation and Specificity of mAbs for TCR $\gamma\delta$. To generate mAb for murine TCR $\gamma\delta$, we immunized Armenian hamsters with anti-CD3 (145-2C11) immunoprecipitate of the lysate of the $\gamma\delta$ T hybridoma KN6. The splenocytes from a hamster whose serum precipitated TCR $\gamma\delta$ were fused to the myeloma line SP2/0. The supernatants of hybridoma cultures were screened by differential staining of TCR $\gamma\delta$ hybridoma KN6 and its TCR-negative variant. The candidates were further screened by immunoprecipitation and cloned (at least three times), and three hybridoma clones which were positive in both analyses were obtained.

Specificities of the mAbs were determined by staining nine TCR $\gamma\delta$-positive T hybrids (18, 19). The results are summarized in Table 1. mAb 3A10 stains all the hybrids, while 8D6 stained three hybrids (KN6, KN108, and KN102), all expressing TCR variable regions $V_{\gamma 4}$ and $V_{\delta 5}$ on their surface. Since 8D6 does not stain six other $\gamma\delta$ T hybridomas, including KI1 and KN106, which express $V_{\gamma 4}$ $V_{\delta 7}$ and $V_{\gamma 7}$ $V_{\delta 5}$ TCR, respectively, its epitope probably consists of a composite determinant provided by $V_{\gamma 4}$ and $V_{\delta 5}$ regions. mAb 5C10 stained only hybrid KN6, which was the source of the immunogen. It is therefore likely to be clonotypic. None of the three mAbs stained the fusion partner BW5147.

Staining properties of the three mAbs just described were consistent with their abilities to immunoprecipitate the characteristic γ and δ polypeptide chains from the Triton X-100-solubilized lysates prepared from the various $\gamma\delta$ T hybrids (Table 1).

To further characterize the binding specificities of the mAbs, the Triton X-100 lysates from KN6 cells were denatured by the addition of $NaDodSO_4$ and dithiothreitol, alkylated with iodoacetamide, and immunoprecipitated with each mAb. mAb 3A10 immunoprecipitated free δ chains but not free γ chains from the KN6 lysate thus prepared (Fig. 1, 3A10, lane 2). Taken together with data summarized in Table 1, these results indicate that 3A10 recognizes a determinant on the constant (C) region of δ chains. Since there seems to be only one C_δ gene segment in mouse (4), 3A10 may be a universal reagent for mouse $\gamma\delta$ TCR. mAb 8D6 precipitated neither the reduced and alkylated γ nor δ (Fig. 1, 8D6, lane 2), consistent with the conclusion that this mAb recognizes a determinant formed by the interaction of $V_{\gamma 4}$ and $V_{\delta 5}$ regions. mAb 5C10 also failed to immunoprecipitate free γ or δ chains.

Table 1. Specificity of mAbs

Cell line	*V* gene segments used		mAb reactivity*					
			3A10		8D6		5C10	
	γ	δ	Fl.	Ppt.	Fl.	Ppt.	Fl.	Ppt.
KN6	4	5	+	+	+	+	+	+
KN108	4	5	+	+	+	+	−	−
KN102	4	5	+	+	+	+	−	−
KI1	4	7	+	+	−	−	−	−
KI129	5	1	+	+	−	−	−	−
V17	5	3	+	+	−	−	−	−
KI21	6	1	+	+	−	−	−	−
KI98	6	1	+	+	−	−	−	−
KN106	7	5	+	+	−	−	−	−
BW5147	None	None	−	−	−	−	−	−

*Fl., cell surface staining by indirect fluorescence analysis; Ppt, immunoprecipitation of antigens solubilized by 1% Triton X-100.

Ontogeny of TCR $\gamma\delta$ and TCR $\alpha\beta$. Using the C_δ mAb 3A10, we quantitated the number of $\gamma\delta$ TCR-bearing thymocytes (abbreviated hereafter as $\gamma\delta$ thymocytes) as a function of age. For comparison, we also determined the number of $\alpha\beta$ TCR-bearing thymocytes by using the TCR β-chain-specific mAb 597 (21). The results are summarized in Fig. 2*A*, and representative data of the two-color flow cytometry obtained with 3A10-biotin conjugate and 2C11 (anti-CD3)-FITC conjugate are shown in Fig. 3*A*.

The total number of thymocytes (data not shown) increased nearly exponentially from day 14.5 to day 18.5 of gestation (referred to as E 14.5, E 18.5, etc), remained at 10^7 cells per thymus until 1 week after birth, and increased rapidly during the next week to reach a plateau of about 2–3 $\times$ 10^8 cells. $\gamma\delta$ thymocytes were detected at E 14.5, the earliest time analyzed (Fig. 2*A*). They increased subexponentially until E 17.5, remained at this level until birth, dipped, and then gradually increased to the adult level of 10^6 cells per thymus. In relative terms, only 0.4–0.6% of total thymocytes at E 14.5 are $\gamma\delta$ thymocytes; this number increases rapidly to its highest value (5%) at E 16.5, then gradually decreases through embryonic life and the first postnatal week until it reaches a stationary adult level of about 0.3–0.5% about 10 days after birth.

mAb 597-positive cells (i.e., $\alpha\beta$ cells) are rare (<100 cells per thymus) at E 14.5 but outnumber $\gamma\delta$ thymocytes after E 16.5. By E 18.5 they are the dominant thymocyte population, their number varying in parallel with the number of total thymocytes.

One additional point is worth noting. As shown in Fig. 3*A*, $\gamma\delta$ thymocytes are anti-C_δ and anti-CD3 "bright" throughout the ontogeny. This is in contrast to $\alpha\beta$ thymocytes, an overwhelming majority of which are known to be dull positive with either CD3 or $\alpha\beta$ mAb (23). This may be partially

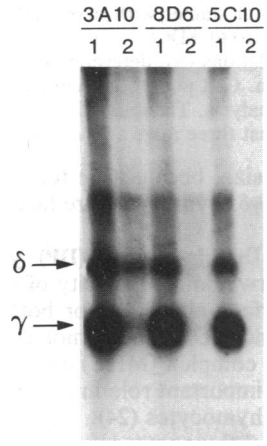

FIG. 1. Immunoprecipitation of γ and δ chains of KN6 cells with three mAbs. Immunoprecipitates from ^{125}I-labeled cell lysates were analyzed by $NaDodSO_4$/PAGE (10% acrylamide) under reducing conditions. Triton X-100 (1%) lysates were directly incubated with mAb-coated protein A-Sepharose 4B beads (lanes 1) or were reduced and alkylated prior to incubation (lanes 2).

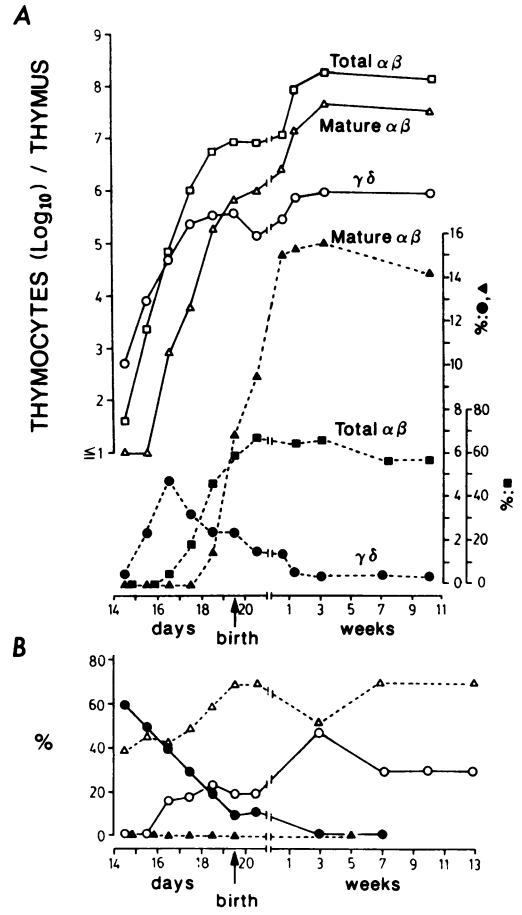

FIG. 2. (*A*) Ontogeny of TCR γδ, αβ thymocytes in C57BL/6 mice. Thymocytes of mice at different ages were stained with anti-δ (3A10) or anti-β (597) biotin conjugates followed by a streptavidin-PE conjugate together with an anti-CD3 (2C11) FITC conjugate. The samples were analyzed with FACScan (Becton Dickinson) by using FACSCAN software. Closed symbols represent the percentages of γδ and total and mature αβ thymocytes among total thymocytes. Mature αβ thymocytes were evaluated as bright cells in CD3-FITC color. Open symbols represent the absolute numbers of cells per thymus estimated from the numbers of total thymocytes and the percentages. (*B*) Differential expression of γδ chains on thymocytes of C57BL/6 mice at different ages. The thymocytes were stained with mAbs 536 (possibly specific for $V_{\gamma 5}$ $V_{\delta 1}$), 8D6 (anti-$V_{\gamma 4}$ $V_{\delta 5}$), and 5C10, in FITC color, and were counter-stained with mAb 3A10 (anti-C_δ) in PE color. The percentages of 536 (●), 8D6 (○), and 5C10 (▲) -positive cells were plotted. Other populations (△), determined by subtraction of those data, are also shown. Cell pools from embryos and newborn mice were used for the analysis. The data of young and adult mice were averaged from at least three mice at each age.

explained by cell size, because the forward light scattering data suggest that γδ thymocytes are larger than αβ thymocytes.

CD4 and CD8 Double-Positive (DP) γδ Thymocytes. In mammals, an overwhelming majority of αβ thymocytes express on their surface either one or both of the accessory molecules CD4 and CD8. These molecules bind to major histocompatibility complex (MHC) class I or class II molecules and play an important role in the recognition of MHC molecules by αβ thymocytes (24).

In contrast to αβ thymocytes, γδ thymocytes have been shown to be enriched in the CD4-negative, CD8-negative (i.e., "double-negative" or DN) subpopulation (6–10, 17). However, since these studies were carried out by immunoprecipitation using anti-γδ sera unsuitable for cell surface staining, a detailed analysis of γδ thymocytes for the expression of the accessory molecules has not been possible. We therefore examined thymocytes from mice of various ages by triple-color staining with C_δ (3A10), CD4 (GK1.5), and CD8 (53-6.7) mAbs. For comparison, CD4 and CD8 expression of thymocytes and the αβ TCR-bearing thymocytes were also examined (Fig. 3*B*). At E 15.5, an overwhelming majority (>85%) of γδ thymocytes were CD4 CD8 DN, and in adult mice, most γδ thymocytes (>60%) are DN. However, at E 16.5, CD4 dull single-positive (SP) and CD8 dull SP γδ T cells appeared, followed by CD4 dull, CD8 bright DP γδ T cells (E 17.5). While the fraction of CD4 dull SP γδ T cells declined after the initial increase, CD8 dull SP, CD4 CD8 dull DP, and CD4 CD8 bright DP cells became the three major γδ-thymocyte subpopulations during late embryonic life (E 18 and after) and the first week after birth. These results are at variance with earlier studies, which implied that the embryonic γδ thymocytes are mostly DN (7, 10). While the majority of γδ thymocytes of adult mice are, as claimed by previous studies (8, 17), CD4 CD8 DN, these new reagents show that CD4 dull SP, CD8 dull SP, and CD4 CD8 DP γδ thymocytes also exist.

As expected for αβ TCR-bearing thymocytes, a majority of 597-positive cells at E 16.5 are dull CD8 SP. However, this staining pattern is drastically altered by E 18, when an overwhelming majority (≈80%) of 597-positive cells are CD4 CD8 DP with the rest being CD4 SP or CD8 SP. The DP cells are αβ dull-positive, while SP cells are αβ bright-positive (23). This pattern is maintained through the rest of development.

No Detectable TCR αβ and TCR γδ DP Cells. Since γ genes are often rearranged in αβ T cells (3, 25) and β genes in γδ T cells (Y. Takagaki and S.T., unpublished data), the two TCRs may be expressed simultaneously on the surface of a single cell at some point during the development of thymocytes. We therefore analyzed the thymocytes from E 16, E 18.5, and 3- to 10-week-old adult mice by using C_δ (3A10) and αβ (597) mAbs in two-color analysis. The results showed that no more than 1% of 3A10-positive cells were also positive with 597 in any of the ages studied. Representative results of E 18.5 and 8-week-old mice are shown in Fig. 4*A*. These results indicate that the αβ and γδ TCR double positive cells are extremely rare, if any, in thymuses, suggesting the existence of a mechanism restricting the surface expression of TCR to only one of the two types.

No Detectable $CD3^+$, αβ TCR and γδ TCR DN Cells. The discovery of TCR γδ made some wonder how many more different types of TCR may exist. Indeed, Chen *et al.* (26) reported an apparent third type of TCR in chickens. This TCR (TCR 3), like TCR αβ (TCR 2) and TCR γδ (TCR 1), is associated with CD3. We therefore examined the possibility that the mouse equivalent of chicken TCR 3 or any other TCR expressed in association with CD3 might be present on some thymocytes. However, our two-color analysis of thymocytes from 4-day- and 8-week-old mice using 2C11 (anti-CD3) mAb and 3A10 plus 597 mAbs showed an insignificant fraction (less than 0.01%) of $CD3^+$ cells that are both αβ TCR- and γδ TCR-negative (Fig. 4*B*).

Age-Dependent Differential Expression of V_γ and V_δ Gene Segment-Encoded TCR on Thymocytes. Some earlier studies demonstrated that thymocytes from mice of different ages express γδ TCR encoded by different combinations of V_γ and V_δ gene segments (18, 27). For instance, a wave of thymocytes bearing TCR γδ encoded by $V_{\gamma 5}$ and $V_{\delta 1}$ gene segments appears early (E 14.5) in embryonic life (18, 27). As this wave subsides another wave of thymocytes bearing a distinct TCR γδ encoded by $V_{\gamma 6}$ and $V_{\delta 1}$ gene segments arises and peaks around birth (18). Furthermore, γδ-thymocytes of adult mice bear yet another and more diverse sets of TCR γδ (19).

Since two of our mAbs (8D6 and 5C10) seem to be specific for two distinct subsets of TCR γδ, we determined the proportion of 8D6- or 5C10-positive thymocytes among total

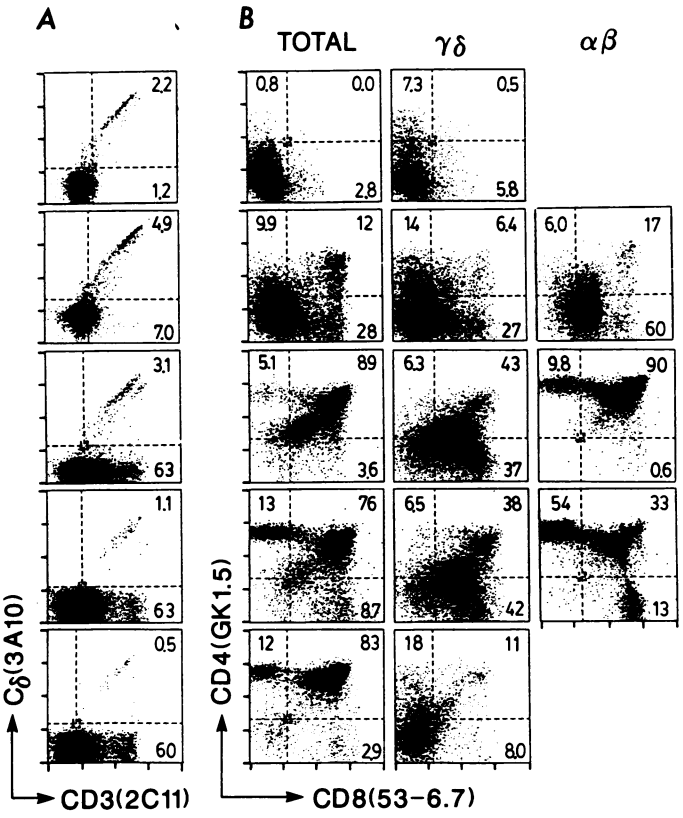

FIG. 3. (A) Two-color immunofluorescence analysis of γδ and CD3 antigens on thymocytes of C57BL/6 mice at different ages. The cells were treated with 3A10-biotin, 2C11-FITC, and streptavidin-PE. Markers indicated by broken lines were settled according to the negative controls incubated with streptavidin-PE and affinity-purified goat anti-hamster IgG-FITC. The analyses were performed as described for Fig. 2; 10,000 events were acquired. The ages of mice are shown on the right of *B*. (*B*) CD4 and CD8 expression on γδ and αβ thymocytes. The thymocytes were stained with anti-δ (3A10) or anti-β (597) conjugates. The cells were further stained with anti-CD4 (GK1.5)-PE and anti-CD8 (53-6.7)-biotin-streptavidin-DuoCHROME. Left column shows the expression of CD4 and CD8 antigens on total populations of thymocytes. Center and right columns show CD4 and CD8 phenotypes of γδ and αβ thymocytes, respectively. The data of γδ- or αβ-positive cells were selectively acquired by gating in FITC color. To confirm the probability of the analysis, E 19.5 γδ sample was purified by cell sorting prior to analysis. Coursor positions were first settled on the background levels of negative controls that had been allowed to react with streptavidin-PE and -DuoCHROME and were further compensated according to the data from indirect immunofluorescence analysis of CD4 and CD8 expression on the total thymocyte populations by using MAR-18-FITC, if necessary. Fluorescence intensities are shown in $\log_{10}$ scales.

γδ thymocytes (measured by mAb 3A10) as a function of the age of mice. For comparison, we also used mAb 536 (27) specific for the $V_{\gamma 5}$-$V_{\delta 1}$ combination. Thymocytes bearing TCR γδ encoded by $V_{\gamma 4}$ and $V_{\delta 5}$ gene segments (i.e., 8D6-positive) started to appear at E 16.5 but stayed at a relatively low level (less than 20% among total γδ thymocytes) during the rest of the embryonic life (Fig. 2*B*). However, such cells constitute a major γδ subset (30–45%) in young adult mice and throughout adulthood. These results are entirely consistent with conclusions drawn earlier on the basis of an analysis of γδ T-cell hybrids (18).

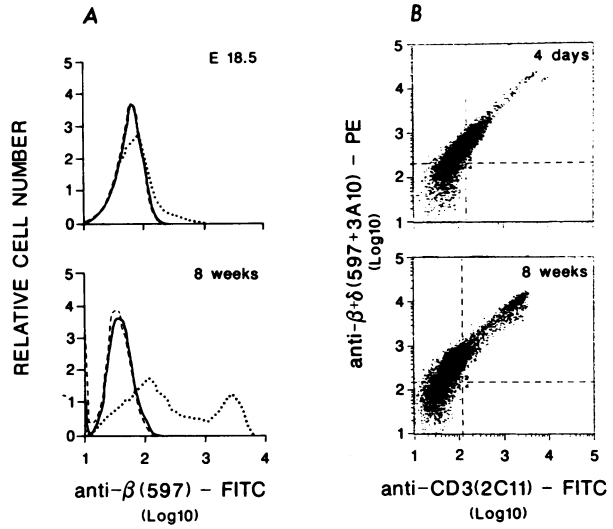

FIG. 4. (*A*) TCR αβ on TCR γδ-positive thymocytes. Thymocytes of fetal (E 18.5) and adult (8-week-old) mice were stained indirectly with anti-β (597) and goat anti-hamster IgG-FITC. After blocking with 10% normal hamster serum, the cells were further stained with anti-δ (3A10)-biotin and streptavidin-PE. —, Green fluorescence of γ-positive cells selected by gating in red color; – – –, data of anti-β-minus controls analyzed in the same way; ·····, anti-β-staining profiles of total thymocytes. (*B*) Correlation of TCR αβ and γδ with CD3 molecules. Thymocytes of 4-day newborn and 8-week adult mice were stained with anti-CD3 (2C11)-FITC together with anti-β (597)- and anti-δ (3A10)-biotin and streptavidin-PE. Analyses were performed as described for Fig. 2.

Fig. 2*B* also shows that thymocytes reactive with the putative clonotypic mAb 5C10 amount to no more than the background level throughout ontogeny. Since mAb 5C10 reacts with a $V_{\gamma 4}$- and $V_{\delta 5}$-encoded TCR, and up to 45% of γδ thymocytes of adult mice bear TCR γδ encoded by this combination of V_γ and V_δ gene segments, the absence of a detectable level of 5C10-positive cells suggests that $V_{\gamma 4}$-$V_{\delta 5}$ TCR are highly heterogeneous.

γδ T Cells in Peripheral Lymphoid Organs. Using mAb 3A10 and other mAbs, we determined the frequency of γδ T cells in peripheral lymphoid organs (i.e., spleen, lymph node, and blood) as well as their CD4 CD8 phenotype. The results are summarized in Table 2. In adult mice (7–13 weeks old), the fraction of γδ T cells among $CD3^+$ T cells is no more than 3% in any of the three sites studied, although it is as high as 10% in the spleen of 4-day-old mice. These results are in contrast with the significantly higher $\gamma\delta^+/CD3^+$ ratio reported for the chicken (29, 30), but they are comparable to frequencies of such cells in humans (1–15%) (31, 32). As the report of Brenner *et al.* (5) on γδ T cells suggested, the majority of peripheral γδ T cells are CD4 CD8 DN (Table 2). However, a significant fraction of γδ T cells is either $CD4^+$ or $CD8^+$ in all the three peripheral lymphoid organs studied. Particularly significant are $CD8^+$ γδ T cells in adult mesenteric lymph node, where almost a third of the total γδ T cells in this organ bear CD8 and there also is a significant level of $CD4^+$ γδ T cells. The fraction of $CD8^+$ cells among the γδ T cells, however, seems to be significantly higher in man (32) and chicken (29, 30). CD4 and CD8 are expressed on γδ T cells at relatively low levels, in contrast to their high level expression on αβ T cells. CD8 expression on peripheral γδ T cells can be induced to a high level by activation with the mAb 3A10 *in vitro* (Y. Utsunomiya, S.I., R.K., S.T., and O.K.,

Table 2. Frequency and surface phenotype of γδ cells in periphery

Organ	Age of animals	No. of animals examined	No. of cells bearing antigen per animal × 10^{-5}			γδ cells among $CD3^+$, %	γδ cells bearing antigen, %		
			CD3	TCR γδ	TCR αβ		$CD4^+$	$CD8^+$	$CD4^-$ $CD8^-$
Spleen	4 days	7*	0.74	0.07	0.67	10	5.5	63	32
	7–13 wk	12	300 ± 73	8.2 ± 2.0	279 ± 70	2.8 ± 0.5	10 ± 0.6	15 ± 4.8	75 ± 5.4
Mesenteric lymph node	7–13 wk	12	51 ± 23	1.2 ± 0.5	50 ± 22	2.3 ± 0.3	7.6 ± 2.6	32 ± 14	60 ± 16
Blood	7–13 wk	17*	25†	0.6	24	2.4	10	18	72

Results are presented as mean ± SD.
*Cells from the indicated number of animals were pooled.
†Estimated from the data in *Biology of the Laboratory Mouse* (28).

unpublished data). Finally, expression of CD4 and CD8 on γδ T cells is drastically different in the spleens of newborn mice. For instance, at the fourth day after birth, over 60% of splenic γδ T cells are CD8 dull-positive, while only 31% are CD4 CD8 DN and a mere 5.5% are $CD4^+$. This pattern of CD4 and CD8 expression on splenic γδ T cells is very like that on γδ thymocytes from newborn mice (Fig. 3*B*).

CONCLUSION

The use of mAbs directed to native epitopes of mouse TCR γδ and other T-cell surface markers allowed us to quantitate and characterize γδ T cells in thymuses of fetal and adult animals as well as in peripheral lymphoid organs. The results confirm some previously drawn conclusions in a more quantitative fashion, but they also upset some widely accepted notions and further characterize T-cell ontogeny. The points of note are as follows: (*i*) In ontogeny, γδ thymocytes appear a few days earlier than αβ thymocytes. (*ii*) Unlike the majority of αβ thymocytes which are TCR dull positive, most γδ thymocytes are TCR bright positive. (*iii*) The CD4/CD8 expression of γδ thymocytes shifts dramatically during the first postnatal week. Before this period, CD4 CD8 DP and CD8 dull SP cells constitute over 80% of γδ thymocytes, while about 70% of γδ thymocytes are DN in the adult thymus. (*iv*) TCR αβ and TCR γδ DP cells are scarce, if present at all, throughout thymic ontogeny. (*v*) Unlike chicken, mouse does not appear to have non-αβ, non-γδ $CD3^+$ T cells. (*vi*) Thymocyte subsets bearing TCR γδ encoded by different combinations of V_γ and V_δ gene segments appear in waves during the thymic ontogeny. (*vii*) While the majority of the γδ T cells in the spleen, mesenteric lymph nodes, and blood are CD4 CD8 DN, $CD8^+$ or $CD4^+$ γδ T cells do occur.

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