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Immunology

Limited diversity of T-cell receptor γ-chain expression of murine Thy-1$^+$ dendritic epidermal cells revealed by V_γ3-specific monoclonal antibody

(T-cell antigen receptor/epidermal cells/mouse)

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ABSTRACT **To study the origin of and the degree of T-cell antigen receptor (TCR) diversity of Thy-1$^+$ dendritic epidermal cells (Thy-1$^+$ dECs) in mice, we have developed a monoclonal antibody (mAb 536) to the γδ TCR. mAb 536 binds to and stimulates interleukin 2 secretion from Thy-1$^+$ dEC but not cells that express TCR composed of α and β chains. mAb 536 precipitates CD3-associated γ and δ chains from lysates of radioiodinated Thy-1$^+$ dECs. Analysis of a panel of hybridomas that express γδ TCR indicated that mAb 536 defines an epitope of the variable region (V_γ3) gene product. Flow cytometric analysis revealed that expression of V_γ3 in the adult mouse is restricted to cells in the epidermis, where essentially all Thy-1$^+$ cells are $V_\gamma3^+$. The majority of CD3$^+$ cells in the 14-day fetal thymus also express V_γ3. These results indicate that the T-cell complement in epidermis are cells that express γδ TCR and that the diversity of antigens recognized by the cells might be restricted by the use of a single V_γ gene segment. Finally, the data raise the intriguing possibility that Thy-1$^+$ dECs may arise from precursors that are among the first to emerge from the developing thymus. This suggests that V gene usage during thymocyte development is highly regulated and has important consequences on the tissue localization and function of the emerging cells. As in other developing tissues, it appears that programmed and transient gene expression determines the fate of the emerging cells.**

Two types of CD3-associated heterodimeric proteins encoded by rearranging antigen receptor genes are found on T cells in lymphoid organs. The vast majority of T cells express receptors composed of clonally distributed αβ heterodimers (1). These T cells generally express the accessory molecules CD4 or CD8 and are capable of specific recognition of antigen in the context of products of the major histocompatibility complex. The second type of T cells express heterodimers composed of the products of the γ (2–5) and δ (6–10) loci and generally do not express CD4 or CD8. The function of this second type of T cell has not been established, although it has recently been suggested that they are capable of recognition of the major histocompatibility complex (11, 12).

It has recently been shown that Thy-1$^+$ dendritic cells present in the skin of mice (dECs) express CD3-associated heterodimers encoded by the T-cell antigen receptor (TCR) γ and δ genes (7, 13–16). Thy-1$^+$ dECs are capable of functions associated with conventional αβ T cells, including direct cytotoxic destruction of cells when targeted via lectins or anti-CD3 antibodies and interleukin 2 (IL-2) secretion upon activation (17). These facts raise the possibility that these cells might be capable of antigen-specific recognition and provide an important component of the immunological armamentarium in the skin. It is therefore of considerable interest to determine the origin of and to assess the degree of potential TCR diversity in these cells. In this report, we describe the production of a monoclonal antibody (mAb) to the γδ heterodimer of Thy-1$^+$ dECs that detects an epitope of the variable region (V_γ3) gene product. We show that the vast majority of Thy-1$^+$ dECs express V_γ3 and may arise from thymic precursors present only during the early stages of thymocyte development. In addition, it appears that the epidermis is the province of cells bearing V_γ3 receptors, since V_γ3 expression is not detectable on cells in the lymphoid tissues of adult mice. These results suggest that thymic differentiation may involve the sequential appearance of distinct cell populations, which emigrate in waves to seed particular tissues. Thus, as is the case in other developing tissues, the regulated and transient expression of specific genes in the thymus has profound effects on the ultimate tissue localization and function of emerging thymocytes.

MATERIALS AND METHODS

Animals. C57BL/6, BALB/c, and AKR mice were obtained from The Jackson Laboratory and from MTS (San Diego, CA). Fetal thymocytes were obtained from timed pregnant BALB/c and C57BL/6 mice. Plug day is designated day 0. Syrian hamsters were obtained from Simonsen Laboratories (Gilroy, CA).

Cell Lines. dEC clones 7-17, 6G3, and 1D2 were independently derived from the skin of AKR mice and are γδ TCR$^+$ (18). D10.G7 (D10) and C6VL-B are αβ TCR-bearing cell lines (19, 20). Hybridomas DN 7.1, DN 7.3, DN 12.1, 33BTE-53.2, 33BTE-67.1, 33BTE-125.1, and 33BTE-140.9 are γδ TCR$^+$ (ref. 21; W.B., unpublished data).

Antibodies. 500A2 is a hamster mAb produced in this laboratory directed against the murine CD3ε chain (22). An antiserum to the human constant region (C_δ) gene product was produced by the immunization of a rabbit with residues 159–187 of the human C_δ gene product (which corresponds to murine residues 162–190) (7). An antiserum directed against C_γ was produced by immunization with a heptapeptide

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Abbreviations: dEC, dendritic epidermal cell; mAb, monoclonal antibody; TCR, T-cell receptor for antigen; IL-2, interleukin 2; V, variable region; C, constant region; J, joining region; ARS, arsenyl derivatized; FITC, fluorescein isothiocyanate; PE, phycoerythrin; APC, allophycocyanin; NP-40, Nonidet P-40.

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composed of residues common to the carboxyl terminus of $C_\gamma 1$–3 (13).

FACS Analysis. Cells (1×10^6) were incubated with saturating amounts of unlabeled, biotin conjugated, or arsenyl derivatized (ARS) mAb for 1 hr at 4°C. Alternatively, antibodies directly conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) were incubated with cells for 1 hr at 4°C. After washing, FITC, PE, or allophycocyanin (APC) coupled avidin, anti-ARS, or anti-immunoglobulin were added to the cells and allowed to incubate an additional hour at 4°C. Cells were analyzed on a FACS 440 (Becton Dickinson Immunocytometry Systems) equipped with an argon ion laser (488 nm) and a helium neon (633 nm) laser. Data analysis was performed using the Consort 30 system (Becton Dickinson).

Immunization and Generation of Hybridomas. A Golden Syrian hamster received three intraperitoneal injections of 1×10^7 7-17 dECs. Three days after the last injection, the spleen was removed and fused with the Ag8.653 murine myeloma line. A modified version of the standard polyethylene glycol method was used for fusion (23). Wells containing actively growing cells after 7–14 days in selective medium were screened for antibody production. Cells from positive wells were repetitively cloned by limiting dilution in the presence of irradiated (1200 rad; 1 rad = 0.01 Gy) rat thymocyte feeder layers. Clones were then retested and expanded to generate large volumes of mAb-containing supernatant.

Immunoprecipitation and PAGE Analysis. Cells were radioiodinated and solubilized in lysis buffer containing 5 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate or 1.5% Nonidet P-40 (NP-40) (20). Immunoprecipitation and PAGE analysis were performed as described (20). For sequential immunoprecipitation, mAb 536-associated components were eluted by reduction and alkylation and then reprecipitated with anti-γ and anti-δ antiserum (7).

Lymphokine Assays. Anti-CD3 (mAb 500A2) and anti-$\gamma\delta$ TCR (mAb 536) antibodies were immobilized on microtiter plates for stimulation of dEC clones as described (17, 24). IL-2 was measured using proliferation of the IL-2-dependent line CTLL-20 (25, 26). IL-2 activity was expressed in units/ml based upon comparison with the National Cancer Institute Biological Response Modifiers Program reference reagent (human Jurkat IL-2, lot ISDP-841).

Preparation of Fresh dEC. Epidermal cell suspensions were prepared as described (27). Briefly, animals were sacrificed and abdominal and dorsal skin was excised and cut into strips and placed in 0.3% trypsin/GNK buffer. After 12–14 hr of incubation at 4°C, epidermis was separated from dermis and treated with fresh trypsin and DNase for 10 min at 37°C. Cell suspensions were subjected to Ficoll/Hypaque separation and Thy-1^+ interface cells were collected after FACS sorting.

RESULTS

Production of mAb to Thy-1^+ dECs. To facilitate detection of antibodies to the $\gamma\delta$ antigen receptor complex in the primary screen hybridoma supernatants were assayed for the capacity to stimulate IL-2 secretion by dEC clone 1D2 cells but not C6VL, which expresses the $\alpha\beta$ TCR. One culture, designated mAb 536, with this property was repetitively cloned and a stable hybridoma was obtained. As shown in Table 1, mAb 536 stimulated production of IL-2 by three dEC clones, 7-17, 1D2, and 6G3, but not C6VL or D10, which express $\alpha\beta$ TCR. Anti-CD3 mAb stimulated lymphokine secretion by all the cell lines. Flow cytometric analysis revealed that mAb 536 bound to each of two dEC clones but not to the lines that expressed $\alpha\beta$ TCR (data not shown). This differential reactivity of mAb 536 raised the possibility that the antibody was reactive with an epitope of the $\gamma\delta$ heterodimer.

Table 1. Stimulation of IL-2 release by mAb 536

Cells	IL-2, units/ml: CD3	IL-2, units/ml: mAb 536
7-17	36*	38
1D2	28	26
6G3	24	30
C6VL-B	45	0
D10	54†	0

Cells ($1–5 \times 10^4$) were added to wells coated with anti-CD3 or mAb 536. Supernatants were collected after 24 hr of culture and tested for the presence of IL-2.

*The lymphokine produced by 7-17, 1D2, and C6VL is most likely IL-2, since IL-2 mRNA is induced in these cells after activation.

†The lymphokine produced by the D10 clone is IL-4, which is detected in the IL-2 assay.

mAb 536 Detects an Epitope of the $\gamma\delta$ Heterodimer. A series of immunoprecipitation experiments was performed to define the structure reactive with mAb 536. In 1D2 lysates obtained with 5 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate in which the CD3–heterodimer association remains intact, mAb 536 and anti-CD3 yielded essentially identical SDS/PAGE profiles under both reducing and nonreducing conditions. In lysates obtained with NP-40, which dissociates CD3 and the TCR heterodimer, mAb 536 yielded a single band with the mobility of the heterodimer under nonreducing conditions and bands of 52 and 42 kDa under reducing conditions. Taken together, these results indicate that mAb 536 does not react with CD3, but it is reactive with epitopes on the heterodimer of 1D2 cells.

Antisera to synthetic peptides corresponding to sequences of the γ and δ chains were used to determine the identity of the proteins reactive with mAb 536. Precipitates obtained from 1D2 cells with mAb 536 in the presence of NP-40 were eluted, reduced, alkylated, and reprecipitated with the peptide antisera. As shown in Fig. 1, precipitation with anti-γ yielded the 42-kDa band, while anti-δ yielded the 52-kDa component. These results provide definitive support for the conclusion that mAb 536 is reactive with the $\gamma\delta$ heterodimer. Efforts to use a similar approach to determine the identity of the chain reactive with mAb 536 were unsuccessful, perhaps as a result of irreversible denaturation of the epitope during the processes of elution, reduction, and alkylation.

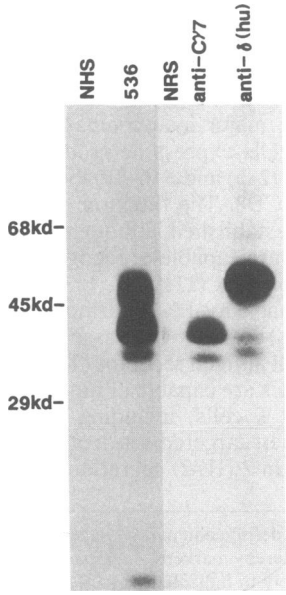

FIG. 1. mAb 536 immunoprecipitates a CD3-associated heterodimer from Thy-1^+ dEC clone 7-17 consisting of γ and δ chains. Antigen immunoprecipitated by mAb 536 was eluted with SDS, reduced and alkylated, renatured by dilution in NP-40, and reprecipitated with antisera to the TCR γ (anti-$C_\gamma 7$) or δ (anti-δ) chains.

Table 2. Reactivity of $CD4^-8^-$ hybridomas with mAb 536

Cells	TCR genes	mAb 536
33BTE-53.2	$V_\gamma3$ V_δ (not $V_\delta1$)	+
33BTE-67.1	$V_\gamma2$ V_δ (not $V_\delta1$)	−
33BTE-125.1	$V_\gamma2$ V_δ (not $V_\delta1$)	−
33BTE-140.9	$V_\gamma4$ $V_\delta1$	−
DN 7.1	$V_\gamma4$ $V_\delta1$	−
DN 7.3	$V_\gamma2$ $V_\delta5$	−
DN 12.1	$V_\gamma4$ $V_\delta1$	−

Hybridoma cells (10^6) were incubated with saturating amounts of mAb 536 culture supernatant followed by fluoresceinated rabbit anti-hamster immunoglobulin. Cells were analyzed on a FACS 440 (Becton Dickinson) and were considered positive when binding was greater than that with control mAb-stained cells.

The Epitope Defined by mAb 536 Is Related to the $V_\gamma3$ Gene Product. We have previously determined by Northern blot and sequence analysis that each of the dEC clones expresses δ chains encoded by a rearranged $V_\delta1$–$D_\delta2$–$J_\delta2$–C_δ (D, diversity region; J, joining region) combination and γ chains encoded by the $V_\gamma3$–$C_\gamma1$ gene (7, 13, 28). To determine the origin of the epitope defined by mAb 536, a panel of well-characterized hybridomas that express $\gamma\delta$ TCR were examined for expression of the mAb 536-reactive epitope by flow microfluorometry. The hybridomas, including representatives that shared expression of either V_γ or V_δ with the dEC clones as well as representatives that expressed distinct V_γ and V_δ gene segments, are described and the results of the immunofluorescence analysis are presented in Table 2. The fact that mAb 536 was unreactive with hybridomas DN 7.1, DN 7.3, DN 12.1, 33BTE-67.1, 33BTE-125.1, or 33BTE-140.9 indicates that the antibody does not detect monomorphic epitopes of the TCR γ or δ chains. The failure of mAb 536 to react with hybridomas DN 7.1, DN 12.1, or 33BTE-140.9, which like the dEC clones express δ chains encoded in part by the $V_\delta1$ gene segment, suggest that the target is not the δ chain. mAb 536 does react with hybridoma 33BTE-53.2, which shares with the dEC clones expression of a γ chain encoded in part by the $V_\gamma3$ segment, but differs in its V_δ usage. Taken together, these data provide strong support for the conclusion that mAb 536 defines an epitope of the $V_\gamma3$ gene product.

$V_\gamma3$ Is Not Expressed by Significant Numbers of Cells in the Adult Lymphoid Organs but Is Readily Detectable on Cells in the Early Fetal Thymus. To determine the distribution of cells expressing $V_\gamma3$ in adult lymphoid organs, cells harvested from the thymus, spleen, and mesenteric lymph nodes of adult AKR, C57BL/6, and BALB/c mice were subjected to flow cytometric analysis using anti-CD3 and mAb 536. The data for AKR are presented in Fig. 2. Cells reactive with mAb 536 were undetectable (<1%) in these organs. Similar results were obtained for C57BL/6 and BALB/c cells. To restrict the analysis to $CD3^+$ cells, two-color immunofluorescence was also performed. As shown in Fig. 2, there was again no evidence of cells that expressed $V_\gamma3$. Similar results were obtained with cells from BALB/c and C57BL/6 mice. These results indicate that cells expressing $V_\gamma3$, if present in the adult lymphoid organs, represent a very small subpopulation of cells.

It has been previously demonstrated that cells expressing the $\gamma\delta$ TCR are $CD4^-8^-$ (5). To further increase the level of sensitivity of our analysis, $CD4^-8^-$ thymocytes and T splenocytes from normal adult BALB/c mice were analyzed by three-color immunofluorescence. As shown in Table 3, there was no evidence of the presence of cells reactive with mAb 536 in the enriched populations. These results indicate that $V_\gamma3^+$ cells are absent or represent a vanishingly small fraction of $CD3^+$ cells, which presumably express $\gamma\delta$ TCR, in the $CD4^-8^-$ population of adult splenocytes or thymocytes.

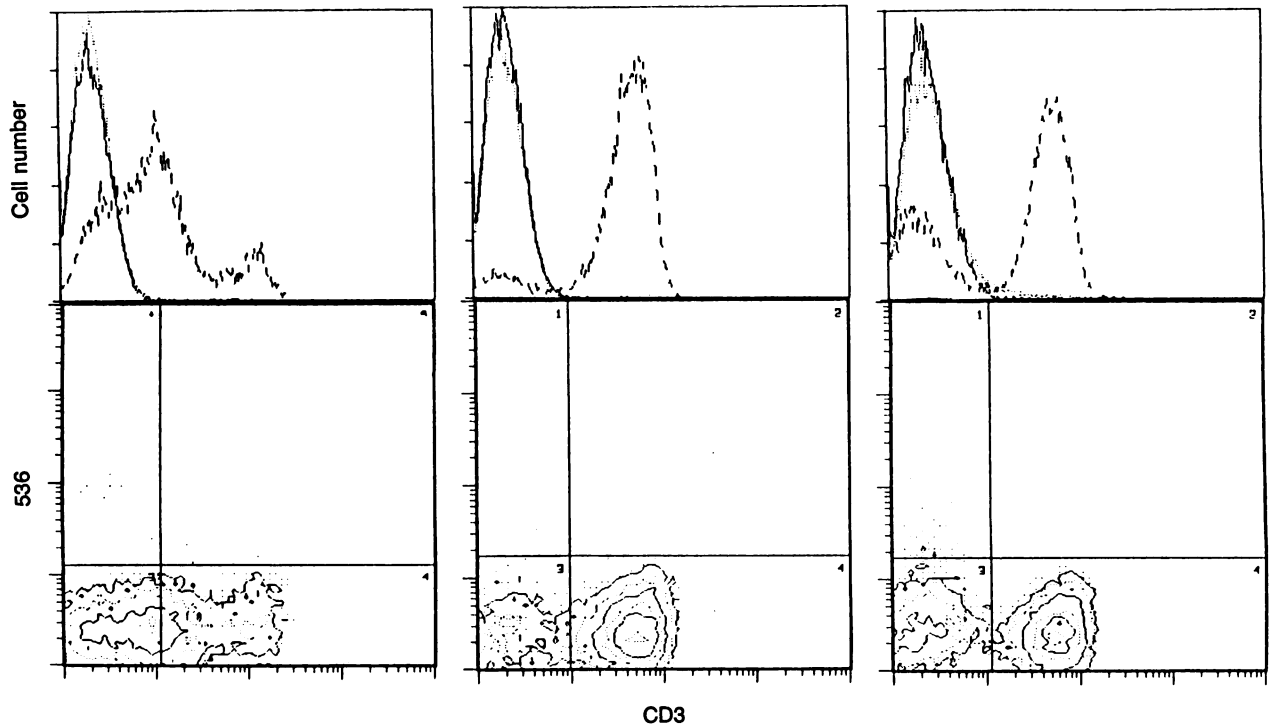

FIG. 2. Cells that express $V_\gamma3$ are not detectable in adult lymphoid organs. Thymus (*Left*), spleen (*Center*), and mesenteric lymph nodes (*Right*) were obtained from 4-week-old AKR mice and live lymphoid cells collected after Ficoll/Hypaque centrifugation. Splenocytes were enriched for T lymphocytes by incubating the cells on goat anti-mouse IgG-coated flasks for 1 hr. The nonadherent cells were 93% Thy-1^+. (*Upper*) Cells were stained as described in Fig. 1. Cells were stained with PE–anti-CD3 (mAb 500A2) ---, FITC–anti-$V_\gamma3$ (mAb 536)——, or control mAb ···. (*Lower*) Data are presented as two-color contour plots. Quadrants were set using the Consort 30 system (Becton Dickinson) based on negative control samples.

Table 3. Reactivity of normal tissue with mAb 536

Cells	% $CD3^+$ cells that are mAb 536^+
Adult thymus*	<0.4
Adult T splenocyte	<0.3
Adult lymph node	<0.8
Adult $CD4^-8^-$ thymus†	<0.01
Adult $CD4^-8^-$ T splenocyte	<0.03
Day-14 fetal thymus‡	92
C57BL/6 dEC (Thy-1^+)§	86
BALB/c dEC (Thy-1^+)	98
AKR dEC (Thy-1^+)	94

*AKR thymus, T splenocyte, and lymph node cells were analyzed by two-color immunofluorescence with FITC–anti-CD3 and ARS–mAb 536, followed by PE–anti-ARS. Similar results were obtained with cells from BALB/c and C57BL/6 mice.

†BALB/c thymus and T splenocytes were analyzed as described in the text.

‡Thymuses were obtained from day-14 BALB/c embryos and were analyzed as described for adult thymus.

§Freshly isolated dECs were analyzed by three-color immunofluorescence with biotin–Thy-1, ARS–mAb 536, and FITC–anti-CD3, followed by APC–avidin and PE–anti-ARS. Thy-1^+ cells were 98% $CD3^+$.

In contrast to the adult lymphoid organs, cells reactive with mAb 536 were readily detectable in the thymus of 14-day embryos. A contour map of two-color analysis of BALB/c embryonic thymus with anti-CD3 and mAb 536 is presented in Fig. 3*A*. Similar results were obtained with C57BL/6 embryonic thymus. It is apparent from the two-color contour map that the majority of the $CD3^+$ cells were also reactive with mAb 536.

The numerical data for the distribution of mAb 536-reactive cells in the various tissues is presented in Table 3. It is apparent that expression of $V_\gamma3$ is rare among cells in the adult lymphoid populations, but that cells expressing $V_\gamma3$ comprise a major component of the $CD3^+$ cells in the early fetal thymus.

The γδ Epitope Defined by mAb 536 Is Expressed by Essentially All Thy-1^+ dECs in Murine Skin. Our initial characterization revealed that Thy-1^+ dEC clones 7-17 and 1D2, which were derived in independent experiments, were both reactive with mAb 536. We next sought to assess the distribution of the epitope on Thy-1^+ dECs in the epidermis of AKR, BALB/c, and C57BL/6 mice. Fresh dEC preparations were obtained; reacted with anti-Thy-1, anti-CD3, and mAb 536; and analyzed by flow microfluorometry. Electronic gates were set to resolve Thy-1^+ cells, and data were displayed as two-color contour plots of CD3 and mAb 536. As shown in Fig. 3*B* and Table 3, essentially all Thy-1^+ cells from all three strains were found to be reactive with both anti-CD3 and mAb 536. These results indicate that expression of $V_\gamma3$ is a common feature of Thy-1^+ dECs in the skin and not a result of random chance or selection by *in vitro* cultivation in the generation of clones.

DISCUSSION

We have previously demonstrated that the antigen receptor expressed by four of four independent dEC clones contains a γ chain encoded in part by $V_\gamma3$ (7, 13). This observation is significant for two reasons. First, the expression of a common V_γ gene by independent clones suggests that the antigen receptor repertoire, at least of the γ chain, might be quite restricted in the Thy-1^+ dEC population. Second, while $V_\gamma3$ transcripts are abundant in the fetal thymus, the level decreases to undetectable levels by birth (29), and there is no evidence of expression of γ chains encoded by $V_\gamma3$ by cells in the lymphoid organs of adult mice. This suggests that Thy-1^+ dECs in adult mice might arise from precursors present only very early in ontogeny. It was of course possible that the small panel of dEC clones, either as a result of chance or of selective pressures occurring during *in vitro* cultivation, present a biased complement of TCRs. We therefore sought to extend our studies by analyzing lymphoid cell populations and freshly isolated dECs for surface expression of $V_\gamma3$.

To facilitate this analysis, we produced a series of mAbs to cell-surface structures of dEC clones. One of these, mAb 536, binds to and stimulates IL-2 production by γδ TCR-expressing Thy-1^+ dEC clones. mAb 536 precipitates the γδ heterodimer from lysates of radioiodinated dEC clones but is unreactive with allogeneic or syngeneic lines that express αβ TCR. These facts, together with the selective reactivity of mAb 536 with a hybridoma that shares with the dEC clones expression of only $V_\gamma3$, indicate that the antibody defines an epitope of the $V_\gamma3$ gene product.

Our finding that the vast majority of Thy-1^+ dECs freshly isolated from the trunk epidermis of adult BALB/c, AKR, and C57BL/6 mice react with mAb 536 suggests that T cells expressing the γδ TCR, rather than αβ, constitute the primary T-cell component in the epidermis. In addition, our results indicate that the γ chains of the TCRs of these cells are largely restricted to usage of one germ-line element, $V_\gamma3$. Biochemical evidence has been reported indicating diversity in the γδ TCR of dEC lines and hybridomas derived from ear epidermis of C3H/HeN (14, 16). The differences may be due to strain differences in $V_\gamma3$ utilization, to the tissues used for isolation of dECs, or to selective processes occurring during isolation and propagation of the cell lines used in those studies.

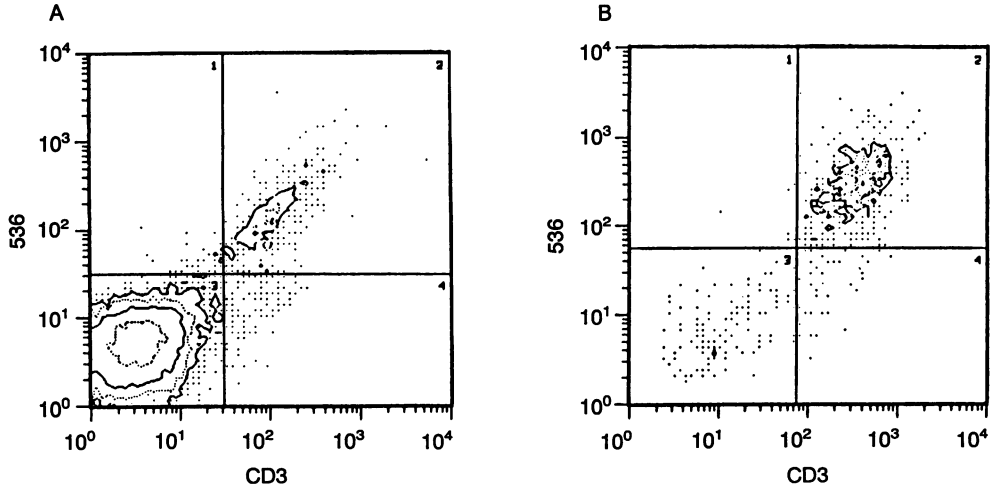

FIG. 3. Essentially all $CD3^+$ early fetal thymocytes and Thy-1^+ dECs express $V_\gamma3$ (*A*) Thymocytes were obtained from day-14 BALB/c embryos. (*B*) Epidermal cells were isolated from the skin of BALB/c mice as described in the text. Three-color FACS analysis was performed with biotin–Thy-1, FITC–anti-CD3 (mAb 500A2), and ARS–anti-$V_\gamma3$ (mAb 536) followed by APC–avidin and PE–anti-ARS. The dEC cell preparation contained 23% Thy-1^+ cells. Analysis of the Thy-1^+ population is presented as a contour display of the binding of CD3 and $V_\gamma3$ mAbs.

It is apparent that, at least in the strains examined, Thy-1$^+$ dECs do not utilize the germ-line diversity available in the V_γ repertoire. As a consequence dEC TCR diversity is limited to that generated in the V–J junctions during rearrangement of the $V_\gamma 3$ gene segment and to that in the δ chain. However, we have failed to detect any junctional variability in the productively rearranged $V_\gamma 3$ or V_δ genes of five dEC clones (28). This lack of variability suggests that the TCR repertoire of dECs may be extremely restricted and that the dEC population may not possess the capability of responding to diverse antigens to the same degree as the clonally elaborated $\alpha\beta$ and $\gamma\delta$ receptors of T cells in the circulation and lymphoid tissues. The restricted antigen receptor repertoire may reflect a focusing of the dEC cells on common antigens or MHC products induced by damage to cells in the epidermis rather than on antigens carried by the agents causing the damage.

While it has been established that Thy-1$^+$ dECs are derived from bone marrow precursors (30), the role of the thymus in generation of functional Thy-1$^+$ dECs is a matter of some controversy. It has been suggested that the cells might arise in the skin from precursors that do not pass through the thymus (30, 31). Thy-1$^+$ dECs are present in the skin of athymic *nu/nu* mice but are abnormal in that TCR γ and δ genes are not expressed and the cells do not express CD3 (32). However, since the skin of *nu/nu* mice also exhibits abnormalities that might impair the development of Thy-1$^+$ dECs in that tissue, it is not possible to attribute the abnormalities of the cells to the absence of the thymus. It has been demonstrated that Thy-1$^+$ dECs reappear slowly (23 weeks after bone marrow grafting) in the skin of radiation chimeras, raising the possibility that precursors may be present in adults (30). However, the cells were not examined for expression of CD3 and may, like Thy-1$^+$ dECs in *nu/nu* skin, be defective for antigen receptor expression. While it remains to be demonstrated, our finding that cells expressing $V_\gamma 3$ are undetectable in the adult thymus, but that the majority of CD3$^+$ cells in the early (14 day) fetal thymus are $V_\gamma 3^+$, raises the intriguing possibility that Thy-1$^+$ dECs may arise from thymic precursors. The first TCR-expressing cells to emerge from the developing thymus might home to the skin and comprise the first component of the T-cell system to appear in ontogeny.

This possibility has two important implications. First, the cells must be very long-lived. Second, the V–J junctions of the Thy-1$^+$ dEC TCR may not contain N regions (33) generated by the action of terminal deoxynucleotidyltransferase (TDT) during rearrangement, since TDT activity is low or absent in the early fetal thymus (34, 35). Our analysis of the junctional sequences of rearranged γ and δ genes in five dEC clones derived from adult mice revealed that N regions are indeed absent in the γ genes and are absent or minimal in the δ genes, consistent with a fetal origin of the cells (28).

It is striking that expression of $V_\gamma 3$ in adult mice is largely restricted to cells in the epidermis. It is possible that $V_\gamma 3$ plays a role in homing of precursors to the skin. Alternatively, it is possible that restricted usage of $V_\gamma 3$ reflects a focusing of the immune system on antigens or major histocompatibility complex products likely to be encountered in epidermis. It will be informative to determine whether other epithelial tissues contain T cells that express TCR of similarly restricted composition.

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