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Immunology

Thy-1$^+$ dendritic epidermal cells belong to the T-cell lineage

(T-cell receptor/extrathymic T-cell differentiation)

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ABSTRACT **The murine epidermis contains a population of dendritic, Thy-1$^+$ cells (Thy-1$^+$ DEC). Although it is now clear that these cells are of bone marrow origin, extensive morphological, histochemical, and cell-membrane marker studies have not definitively placed them in any known hematopoietic differentiation pathway. Based on the observation that Thy-1$^+$ DEC can be propagated *in vitro* with Con A and interleukin 2, we have established three cell lines (Tehy 184, Tehy 245, and Yety 245) that can be continuously grown in medium with lectin–lymphokine-rich culture supernatants of rat spleen cells. With the exception of the loss of reactivity with anti-asialo-G$_{M1}$ antibodies (Tehy 184 and Tehy 245) and the gain of interleukin 2 receptor expression, the cultured cell lines bear the same surface characteristics as freshly isolated Thy-1$^+$ DEC: Thy-1$^+$, Ly-5$^+$, Lyt-1$^-$, Lyt-2$^-$, L3T4$^-$, Ia$^-$, sIg$^-$. Using Southern and RNA gel blot analysis, we now demonstrate that these Thy-1$^+$ DEC-derived cell lines exhibit various patterns of rearrangements in the gene complexes encoding the T-cell receptor (related) β and γ chains and contain mature and/or incomplete transcripts from the T-cell receptor α- and β-chain genes, as well as transcripts from the receptor-related γ-chain genes. Tehy 184 cells, the only cells containing both mature α- and β-chain transcripts, react positively with the F23.1 monoclonal antibody, which recognizes the product of a subset of T-cell receptor β-chain variable region gene segments. This antibody precipitates a surface protein of 84–88 kDa from these cells that after reduction separates into two 40- to 44-kDa chains, characteristic of Ti α/β heterodimers. These data strongly suggest that Thy-1$^+$ DEC belong to the T-cell lineage and point to the epidermis as a site either of immature thymocyte migration or of extrathymic T-cell differentiation.**

The murine epidermis is composed of a number of different cell types including keratinocytes, melanocytes, Langerhans cells, and a population whose most prominent features are a dendritic configuration and the abundant expression of cell-membrane Thy-1 molecules (1, 2). Although it is now clear that these Thy-1$^+$ dendritic epidermal cells (Thy-1$^+$ DEC) are derived from precursor cells originating in the bone marrow (3, 4), the biological role(s) of these cells is still unknown. Immunolabeling studies revealed that the surface phenotype of Thy-1$^+$ DEC (Thy-1$^+$, asialo-G$_{M1}^+$, Lyt-1$^-$, Lyt-2$^-$, L3T4$^-$, Ia$^-$, sIg$^-$) is thoroughly different not only from that of B lymphocytes and mononuclear Ia-positive dendritic cells but also from that of mature T lymphocytes (5). Rather, it is similar to only a small number of lymphoid cell subsets including certain members of the NK cell family (6), immature thymocytes (7, 8), and neonatal suppressor cells (9). Nixon-Fulton *et al.* (10) and our group (E.T., H.Y., G. Stingl, A. Elbe, K. Wolff, and G. Steiner, unpublished results) have shown that Thy-1$^+$ DEC display vigorous proliferative activity when stimulated with Con A and interleukin 2. We have used this strategy to establish three cell lines (Tehy 184, Tehy 245, and Yety 245) that are free of contaminating epidermal cells (EC) and that have now maintained a uniform and stable surface marker profile (Thy-1$^+$, Ly-5$^+$, Lyt-1$^-$, Lyt-2$^-$, L3T4$^-$, Ia$^-$, sIg$^-$) over 12–15 months *in vitro*. In the present experiments, we have analyzed Thy-1$^+$ DEC-derived cell lines for rearrangement, transcription, and expression of T-cell receptor (TCR) genes and find that these cell lines display different patterns of TCR gene transcription similar to the heterogeneity in TCR gene expression in maturing thymocytes.

MATERIALS AND METHODS

Establishment and Phenotypic Characterization of Thy-1$^+$ DEC-derived Cell Lines. C3H/HeN ear EC obtained by standard trypsinization procedures were enriched for Thy-1$^+$ DEC according to a procedure to be described in detail elsewhere (E.T., H.Y., G. Stingl, and G. Steiner, unpublished results). In brief, this method involves several Percoll density-gradient centrifugations and the removal of Langerhans cells using anti-I-A monoclonal antibodies (mAb) plus complement. In certain experiments (establishment of cell lines Tehy 184 and Tehy 245) the resulting cell suspension containing 70–90% Thy-1$^+$ DEC was also treated with anti-Lyt-1 mAb plus complement. Cells were then cultured at a starting density of 0.5–1 $\times$ 10^6 cells per well in 24-well tissue culture plates (Costar, Cambridge, MA) at 37°C (95% air/5% CO_2) in RPMI 1640 (Biofluids, Rockville, MD) containing 10% (vol/vol) fetal calf serum (Biofluids), 2 mM L-glutamine (GIBCO), 50 μM 2-mercaptoethanol, penicillin (100 units/ml), streptomycin (100 μg/ml), amphotericin B (0.25 μg/ml), gentamicin (50 μg/ml), 25 mM Hepes, 0.1 mM nonessential amino acids, and 1 mM sodium pyruvate (all from GIBCO) (complete medium) supplemented with 10–25% (vol/vol) supernatant from Con A-stimulated rat spleen cells (CAS). Cultures were fed CAS-supplemented complete medium every 2–3 days; substantial proliferative activity was first observed after 5–7 days and continued vigorously over the following months in the absence of Ia-positive accessory cells or other feeder cells. The resulting cell lines were propagated in 75-cm^2 tissue culture flasks (Costar), and their phenotypic profile was monitored at

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Abbreviations: EC, epidermal cell(s); Thy-1$^+$ DEC, Thy-1-positive dendritic epidermal cell(s); TCR, T-cell receptor; CAS, supernatant from Con A-stimulated rat spleen cells; mAb, monoclonal antibody; V, variable; D, diversity; J, joining; C, constant; C_α, C_β, and C_γ, C region of the α, β, and γ chain of the TCR, respectively; FACS, fluorescence-activated cell sorter.

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3-week intervals using either a FACS 440 or a FACS Analyzer (FACS, fluorescence-activated cell sorter) (Becton Dickinson). Antibodies used included, from Becton Dickinson, monoclonal anti-Thy-1.2 (rat IgG2b), anti-Lyt-1 (rat IgG2a), anti-Lyt-2 (rat IgG2a), anti-L3T4 (rat IgG2b), as well as anti-common leukocyte antigen (Ly-5, clone M1/9.3.4.HL.2; rat IgG2a; ref. 11), anti-I-A^k (clone 10-2.16; mouse IgG2b; ref. 12), anti-interleukin 2 receptor antibody (clone 7D4; rat IgMk; ref. 13), and a murine monoclonal IgG antibody, F23.1, specific for a subset of TCR β-chain variable (V) region gene segments (14). Second-step reagents included fluorescein isothiocyanate-conjugated MAR 18.5 (mouse IgG2a; from Becton Dickinson) and fluorescein isothiocyanate-conjugated $F(ab')_2$ goat anti-mouse immunoglobulin (Grub antibodies; Scandic, Vienna).

Preparation of DNA and RNA. High molecular weight DNA was prepared from Thy-1^+ DEC-derived cell lines and C3H/HeN kidney according to a standard procedure (15). Total cellular RNAs were extracted from cell lines, Tehy 184, Tehy 245, and Yety 245, and, for control purposes, from EL4 thymoma cells and from WEHI-231 B-lymphoma cells by homogenization of cell pellets in guanidinium thiocyanate followed by centrifugation through a cushion of CsCl (16).

Hybridization Probes. The probe used to detect α-chain gene expression was the 569-base-pair *Nco* I fragment derived from the constant (C) region of a murine TCR α-chain (C_α) cDNA clone (M. A. Norcross and R.N.G., unpublished observation; ref. 17). The 86T5 cDNA clone that includes 5'-flanking β-chain $D_{\beta 1}$, $J_{\beta 1.3}$, and $C_{\beta 1}$ sequences was used to detect β-chain gene expression and rearrangement (18). (D, diversity; J, joining.) The C region of the γ-chain (C_γ) cDNA probe used encompassed the second half of the *CI* exon, all of exons *CII* and *CIII*, and $\approx$120 base pairs of the 3'-untranslated region (M. Avigan and R.N.G., unpublished observations).

RNA Gel Blot Hybridization. Total cellular RNA (10 μg) from each cell line to be analyzed was electrophoresed on a 1% agarose/formaldehyde gel, transferred to nitrocellulose (Schleicher & Schuell) (19), and hybridized with probes labeled by either nick-translation (20) or by hexamer priming (21) for 16 hr at 42°C in 40% (vol/vol) formamide, 4× SSC, 10 mM Tris (pH 7.5), 1× Denhardt's solution, 10% (wt/vol) dextran sulfate, and denatured salmon sperm DNA at 50 μg/ml. The blots were then washed three times each for 20 min in 2× SSC/0.1% $NaDodSO_4$ at 25°C and three times for 20 min in 0.1× SSC/0.1% $NaDodSO_4$ at 60°C. (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0.) After drying, the filters were exposed at −70°C to Kodak XAR-5 film in the presence of intensifying screens.

Southern Blot Hybridization. Various genomic DNAs (10 μg) were digested with the restriction enzymes *Pvu* II, *Hpa* I, *Hin*dIII, or *Eco*RI, electrophoresed on a 0.7% agarose gel, denatured, and transferred to nitrocellulose as described (22). The filters were then hybridized, washed, and exposed as above.

Cell Surface Labeling, Immunoprecipitation, and $NaDodSO_4$/PAGE. Surface proteins on 10^8 cells were labeled with $Na^{125}I$ by lactoperoxidase-catalyzed iodination (23). Labeled membrane proteins were then extracted with 0.5% Triton X-100 in 0.05 M Tris/0.3 M NaCl, pH 7.6, containing 1 mM phenylmethylsulfonyl fluoride, leupeptin (10 μg/ml), and 10 mM iodoacetamide. For immunoprecipitation, F23.1 mAb (anti-TCRβ; ref. 14) and, for control purposes, 10-2.16 (anti-I-A^k; ref. 12) supernatants were incubated with protein A-Sepharose (Pharmacia) for 4 hr at 4°C. These antibody-coupled beads were then washed and incubated with the cell extract for 1 hr at 4°C with continuous agitation. Immunoprecipitates were then washed three times in the same buffer used for membrane protein solubilization, except that the Triton X-100 concentration was reduced to 0.1%. The precipitated samples were then boiled for 3 min in Laemmli sample buffer (24) containing 2% (wt/vol) $NaDodSO_4$ with or without 5% (vol/vol) 2-mercaptoethanol and then subjected to electrophoresis in 10% polyacrylamide slab gels containing 0.2% $NaDodSO_4$. After electrophoresis, the gel was stained with Coomassie blue, destained, dried, and prepared for autoradiography.

RESULTS

Phenotypic Characterization of Thy-1^+ DEC-Derived Cell Lines. When EC enriched for Thy-1^+ DEC were cultured in CAS-supplemented medium, we observed vigorous proliferation of flask-adherent cells after 5–7 days. After 2–3 weeks of culture, these cells had overgrown any other remaining EC, resulting in three independent cell lines, Tehy 184, Tehy 245, and Yety 245. These cell lines have now been maintained in tissue culture for 12–15 months. Repeated FACS analysis revealed that the three cell lines have the following uniform and stable surface-marker profile: Thy-1^+, Ly-5^+, Lyt-1^-, Lyt-2^-, L3T4^-, Ia^-, sIg^- (results not shown). The phenotypic features of the three cell lines correspond to those of freshly isolated Thy-1^+ DEC or Thy-1^+ DEC *in situ* with two notable exceptions. First, within the first few days of culture, Thy-1^+ DEC giving rise to Tehy 184 and Tehy 245 but not Yety 245 progressively lost anti-asialo-G_{M1} reactivity (E.T., H.Y., G. Stingl, and G. Steiner, unpublished observation). Second, we found that a variable percentage of cultured Thy-1^+ DEC as opposed to Thy-1^+ DEC *in situ* (5) expressed interleukin 2 receptors as defined by mAb 7D4 (13).

Transcription of TCR Genes in Thy-1^+ DEC-Derived Cell Lines. All Thy-1^+ DEC-derived cell lines contained high levels of TCR β-chain gene transcripts (Fig. 1*B*); there existed, however, considerable differences in the ratio of 1.3-kilobase (kb) vs. 1.0-kb β-chain C region (C_β) transcripts between the different cell lines. The larger message species are transcribed from VDJ rearrangements, whereas the smaller species are derived from DJ rearrangements (25, 26) or represent germ-line transcripts (27). Whereas Tehy 184 contained roughly equal amounts of 1.3-kb and 1.0-kb C_β mRNA, Tehy 245 had only immature 1.0-kb C_β transcripts (Fig. 1*B*). The predominant C_β mRNA species in Yety 245 was 1.2 kb long. The exact identity of this 1.2-kb C_β transcript will require nucleotide sequence analysis for resolution. However, it should be noted that a similar-sized transcript in a T-cell hybridoma has been shown by nucleotide sequencing to correspond to a DJC transcript of the *β1* locus (R.I.L. and R.N.G., unpublished observations).

In contrast to the quantitatively similar levels of TCR β-chain mRNA in these various Thy-1^+ DEC-derived lines, the steady-state amounts of α-chain mRNA were found to be quite variable. Similar to EL4 cells, two distinct bands at 1.7 and 1.4 kb were seen when Tehy 184 mRNA was hybridized with the C_α probe (Fig. 1*A*). The 1.7-kb transcript corresponds in size to full-length VJC α-chain mRNA seen in mature T lymphocytes. The 1.4-kb transcripts hybridizing to C_α probes have been noted (17), but their structure has not been reported. In contrast to Tehy 184, Yety 245 contained only the smaller 1.4-kb C_α-hybridizing transcripts discernible only after a 3-week exposure of the autoradiographs (Fig. 1*A*). In Tehy 245 cells, no C_α-containing mRNA was detectable, even after a 3-week exposure of the autoradiographs (Fig. 1*A*).

Of particular interest was the finding that all three Thy-1^+ DEC-derived cell lines exhibited high levels of γ-chain transcripts. As with EL4 cells, only one species of C_γ-containing transcripts was detected in Yety 245, corresponding in size to VJC γ-chain mRNA of 1.6 kb (Fig. 1*C*). In addition to high levels of such 1.6-kb messages, Tehy 184 and Tehy 245 also had 1.3-kb C_γ-containing transcripts; the

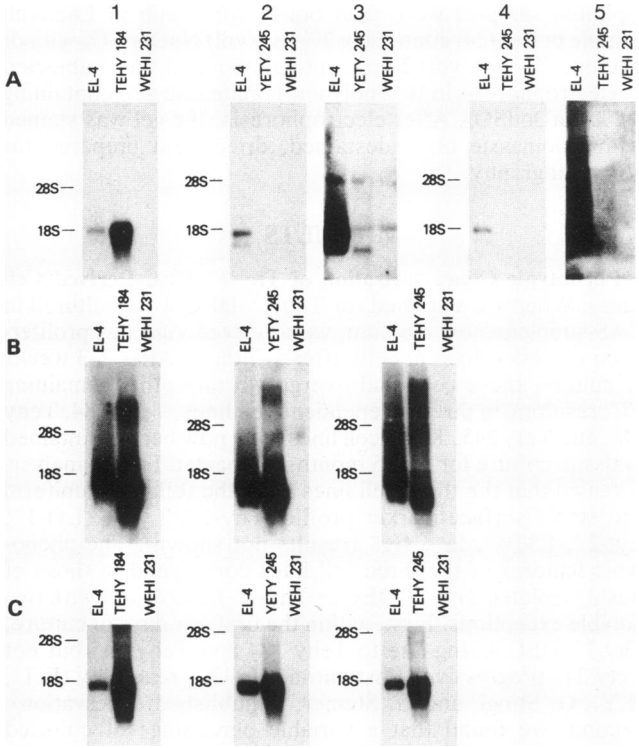

FIG. 1. Expression of T-cell antigen receptor (-related) genes by Thy-1$^+$ DEC-derived cell lines (Tehy 184, Yety 245, and Tehy 245) as compared to EL4 T lymphoma and WEHI-231 B-lymphoma cells. RNA gel blots of total cellular RNA were performed. Each filter contained 10 μg of RNA per lane. Hybridization probes were C_α (*A*), C_β (*B*), and C_γ (*C*). Exposure times were as follows. C_α (*A*): blots 1, 2, and 4 for 24 hr; blots 3 and 5 for 3 weeks. C_β (*B*): all lanes for 3 days. C_γ (*C*): all lanes for 3 days. Ribosomal RNAs (28 S and 18 S) were used as size markers.

nature of these latter C_γ mRNAs has not been clearly defined, but they may correspond to rearrangements of as yet undefined D segments to the J region of the γ chain, or to germ-line transcripts from unrearranged J and C gene segments of the γ-chain locus (M. Avigan and R.N.G., unpublished results).

Rearrangement of TCR Genes in Thy-1$^+$ DEC-Derived Cell Lines. To evaluate the clonality of these cell lines and to look for DNA rearrangements consistent with the observed patterns of TCR mRNA transcription, Southern blot analysis was performed on high molecular weight DNA from Tehy 184, Tehy 245, and Yety 245. This analysis was carried out only with β- and γ-chain probes, as α-chain rearrangements are rarely observed using C_α probes, due to the large distances between C_α and many α-chain J-region gene segments.

For the cell lines Tehy 184 (1.3- and 1.0-kb β-chain mRNA) and Yety 245 (1.2-kb β-chain mRNA), Southern analysis revealed DNA rearrangements of the β locus consistent with the transcripts found in these cells (Fig. 2). After hybridization to the 86T5 β probe, germ-line C3H/HeN kidney DNA digested with *Hin*dIII gave two bands at 3.1 and 9.3 kb. Tehy 184 DNA digested with *Hin*dIII and probed in the same manner showed a loss of the 9.3-kb germ-line band but had a new 7.8-kb band consistent with rearrangement to the $C_{\beta 1}$ locus. Yety 245 retained both germ-line *Hin*dIII bands, but gained an 8.5-kb band consistent with a D to J rearrangement of the $\beta 1$ locus (28). The *Hpa* I-digested germ-line DNA gave bands at 11.6 kb and 6.1 kb when hybridized to the 86T5 probe. Analysis of *Hpa* I digests showed two nongerm-line bands in Tehy 184 DNA at 13 and 9.1 kb, indicating that several rearrangements involving the C regions at both the $\beta 1$ and $\beta 2$ loci have occurred in these cells. These data suggest the possibility of clonal heterogeneity in at least the Tehy 184 line (see below). Hybridization of *Hpa* I-digested Yety 245 DNA with the 86T5 probe gave two bands at 16.6 and 17.2 kb, consistent with two separate rearrangements at the C region of the $\beta 2$ locus.

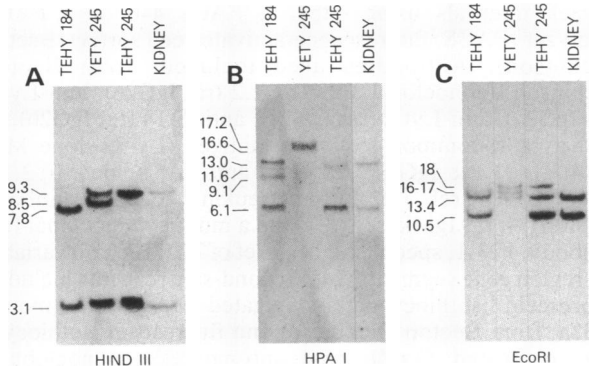

FIG. 2. β- and γ-chain gene rearrangement in Thy-1$^+$ DEC-derived cell lines (Tehy 184, Yety 245, and Tehy 245). DNA from these cell lines and from adult C3H/HeN kidney was digested with *Hin*dIII (*A*), *Hpa* I (*B*), and *Eco*RI (*C*). Southern blots were performed. Each filter contained 10 μg of DNA per lane. Hybridization probes were C_β (*A* and *B*) and C_γ (*C*). Exposure time was 2 days. The approximate sizes of the hybridizing restriction fragments are indicated in kb.

The Southern blot pattern seen using Tehy 245 DNA and the 86T5 probe could not be directly correlated with the mRNA pattern seen on RNA gel blots. Only germ-line bands were seen with blots of *Hin*dIII- or *Hpa* I-digested DNA (Fig. 2). When *Pvu* II was used to digest kidney DNA, a new band of 5.0 kb was observed in addition to the expected 6.3-, 6.1-, and 5.9-kb bands. This band was also seen in the Tehy 184 and Yety 245 DNAs, but not in the Tehy 245 DNA. Although the nature of this β-locus gene segment is not yet known, these findings suggest that either the 1.0-kb transcript in Tehy 245 cells is derived from transcription of an unrearranged C_β segment (27, 29), or that it represents transcription from a DJ rearrangement involving a D segment in the 5.0-kb germ-line band missing in the Tehy 245 DNA.

Blots prepared with *Eco*RI-digested DNA were hybridized to a C_γ probe to examine rearrangements at this locus (Fig. 2). The 13.4- and 10.5-kb bands represent germ-line C_γ segments also seen in the kidney DNA. The absence of the 7.5-kb band present in BALB/c DNA is consistent with the deletion of this pseudogene segment in several mouse strains (30, 31). In addition to germ-line bands, Tehy 184 showed a faint rearranged band at 16–17 kb, whereas Tehy 245 showed a new 18-kb band. Yety 245 had both the 16- to 17-kb and the 18-kb rearranged bands and lacked the germ-line 10.5-kb band. The 18-kb band may represent the V-region rearrangement at the $\gamma 3$ locus, which has been frequently observed in fetal thymocytes but rarely found in more mature T cells (32). The rearrangements resulting in the 16- to 17-kb fragment cannot be determined by the use of a C_γ probe only and will require hybridization with the various γ-chain V-region probes.

mAb F23.1 Reactivity of Thy-1$^+$ DEC-Derived Cell Lines. When tested by FACS analysis with F23.1 mAb directed against an allotypic determinant of the TCR β chain (14), a certain percentage of Tehy 184 cells (containing full-sized TCR α- and β-chain transcripts) exhibited clear F23.1 surface staining (Fig. 3*A*), in contrast to Yety 245 (Fig. 3*B*) and Tehy 245 (Fig. 3*C*), which were consistently unreactive. Immunoprecipitation of radioiodinated surface proteins from Tehy 184 with protein A-Sepharose-bound F23.1 revealed a broad band at ≈43–44 kDa under reduced conditions that migrated

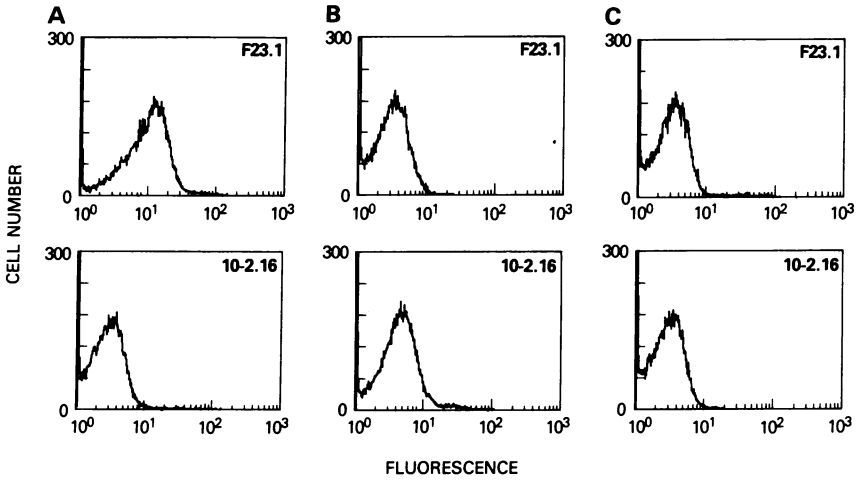

FIG. 3. Reactivity of Thy-1$^+$ DEC-derived cell lines with anti-TCR β-chain antibody F23.1 (14) and, for control purposes, with 10-2.16 (anti-I-A^k; ref. 12). Tehy 184 (*A*), Yety 245 (*B*), and Tehy 245 (*C*) cells (5×10^5 cells) were first incubated with either F23.1 or 10-2.16 for 30 min at 37°C in the presence of 0.2% NaN_3. After three phosphate-buffered saline washes, antibody binding was assessed by using fluorescein isothiocyanate-conjugated F(ab')$_2$ goat anti-mouse immunoglobulin. After three washes, samples were analyzed on a FACS Analyzer. Results are reported in arbitrary units.

at 84–88 kDa under nonreduced conditions, consistent with the typical gel pattern for a TCR α/β dimer (Fig. 4).

DISCUSSION

The data presented in this study on the pattern of rearrangement and transcription of TCR genes in Thy-1$^+$ DEC-derived cell lines (Thy-1$^+$, Ly-5$^+$, Lyt-1$^-$, Lyt-2$^-$, L3T4$^-$, Ia$^-$) and on the presence of TCR α/β heterodimers on the surface of one of these lines provide strong evidence that Thy-1$^+$ DEC belong to the T-lymphocyte lineage. A comparison between Thy-1$^+$ DEC-derived cell lines and established subsets of the T-cell family shows that Thy-1$^+$ DEC-derived cell lines resemble certain NK cell lines, immature intrathymic precursor thymocytes (double-negative cells; Thy-1$^+$, L3T4$^-$, Lyt-2$^-$), and early (day 13–15) fetal thymocytes not only in their phenotypic features but also with regard to the pattern of TCR gene expression. Although Yanagi *et al.* (33) demonstrated that certain Thy-1$^+$, Lyt-1$^-$, Lyt-2$^-$ NK cell lines rearrange and express RNA of the TCR β-chain genes, NK cell activity of a given cell is not dependent upon the expression of functional T3/Ti α/β complexes and may occur in the absence of either functional Ti α- and β-chain transcripts or demonstrable β-chain gene rearrangement (34, 35). Although Nixon-Fulton *et al.* (36) have reported that Con A-stimulated Thy-1$^+$ EC exhibit NK cell-like activity, several attempts to demonstrate Yac-1 cell lysis by our cell lines have so far been negative (H.Y. and G. Stingl, unpublished observations).

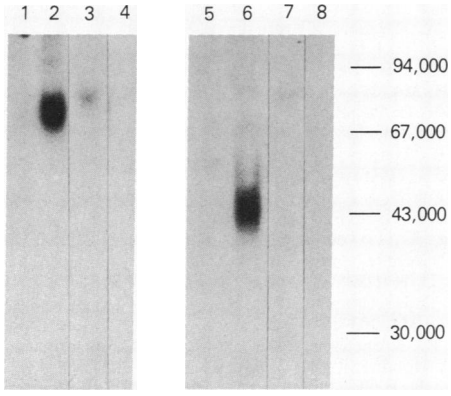

FIG. 4. The monoclonal antibody F23.1 precipitates a molecule with the characteristics of the TCR α/β heterodimer from Tehy 184 cells, but not from Yety 245 cells. Tehy 184 and Yety 245 cells were surface-radioiodinated and were then lysed. The lysates were precipitated with either protein A-Sepharose-bound F23.1 or with protein A-Sepharose-bound 10-2.16. NaDodSO$_4$/PAGE was performed under nonreducing (lanes 1–4) and reducing conditions (lanes 5–8). Lanes: 1 and 5, Tehy 184 lysate precipitated with 10-2.16; 2 and 6, Tehy 184 lysate precipitated with F23.1; 3 and 7, Yety 245 lysate precipitated with 10-2.16; 4 and 8, Yety 245 lysate precipitated with F23.1.

Notwithstanding certain phenotypic differences between resident Thy-1$^+$ DEC and double-negative immature thymocytes [i.e., the latter cells express interleukin 2 receptors *in situ* (37, 38) whereas the former cells do not (5)], the fact that both cell systems reside within a stratified squamous epithelium may be more than a fortuitous coincidence. Although the patterns of α-, β-, and γ-chain expression in the developing fetal thymus are known only for heterogenous populations, it is now clearly established that rearrangement and expression of the various TCR (related) genes occur at different times during T-cell ontogeny (39, 40). Transcription of γ-chain genes, but not α- and β-chain genes, can be detected in day 12 fetal thymocytes (D. M. Pardoll, B. J. Fowlkes, R.I.L., R.N.G., and R. H. Schwartz, unpublished results). The 1.0-kb DJC region of the β- but not the C_α-containing gene transcripts can first be observed on day 13/14 (28, 39–41) (D. M. Pardoll, B. J. Fowlkes, R.I.L., R.N.G., and R. H. Schwartz, unpublished results). Substantial levels of both the 1.3-kb β-chain and 1.7-kb α-chain mature transcripts are not present until day 17 of gestation (39–41); this event apparently correlates with an increase in transcription of the δ subunit of the T3 molecule (40) and with the first appearance of TCR α/β heterodimers on the cell surface (41). Thy-1$^+$ DEC-derived cell lines characterized in this study may well represent analogues to double-negative thymocytes at different stages of maturation. Tehy 245 cells exhibit abundant transcripts of rearranged γ-chain genes, truncated 1.0-kb β-chain transcripts without demonstrable β-chain gene rearrangement, and no α-chain transcripts. This cell line may thus be equivalent to day 13/14 fetal thymocytes. Yety 245 cells contain transcripts of rearranged γ-chain genes, 1.2-kb β-chain transcripts of (incompletely?) rearranged β-chain genes, and only low levels of truncated 1.4-kb α-chain mRNA and may thus correspond to day 15/16

fetal thymocytes and double-negative adult thymocytes. Tehy 184 contain both mature and truncated α-, β-, and γ-chain transcripts of rearranged genes. The further observation that a portion of these cells display F23.1 reactivity and that this antibody precipitates a disulfide-bonded 84- to 88-kDa dimer from surface-labeled Tehy 184 cells strongly suggests that at least a fraction of these cells expresses functional TCR α/β complexes on their surface. The heterogeneity in surface TCR expression of Tehy 184 cells presumably relates to the uncloned state of this cell line.

At the present time, it cannot be decided whether the heterogeneity in TCR gene/protein expression in distinct Thy-1$^+$ DEC-derived cell lines results from maturational changes induced in immature ancestor cells by long-term culture in CAS-supplemented medium, or, alternatively, reflects a heterogeneity of resident Thy-1$^+$ DEC fixed at various maturational stages during the establishment of the *in vitro* lines. Our consistent failure to detect F23.1-reactive cells in epidermal sheets or freshly isolated EC suspensions (G. Stingl, unpublished observation) together with the F23.1 mAb reactivity of a portion of Tehy 184 cells suggests that certain Thy-1$^+$ DEC can be induced to express surface-bound α/β complexes. The fact that a similar differentiation step, i.e., transcription of full-sized α- and β-chain mRNAs, did not occur in the other cell lines can be explained in several ways. First, it may well be that the vast majority of Thy-1$^+$ DEC have a great capacity for self-renewal, but only a limited potential for differentiation. Second, a special microenvironment, either factors other than the ones present in CAS-conditioned medium, or even longer culture periods, may be needed for the occurrence of such an event. Finally, one should consider the possibility that certain Thy-1$^+$ DEC and cell lines derived therefrom, as well as certain double-negative thymocytes, are not simply immature precursors of T cells expressing surface-bound α/β heterodimers but rather represent an alternative T-cell differentiation pathway. It has been shown (42–44) that there are human lymphocytes that express the T3 glycoprotein but not the TCR α and β subunits. Cross-linking experiments revealed that these lymphocytes express T3-associated polypeptides, one of which appears to be the product of the TCR-related γ-chain gene (42–44). We have preliminary evidence that cell lines Tehy 245 and Yety 245, which lack functional α- and β-chain gene transcripts, exhibit surface-bound T3 antigens. This T3$^+$ Tiα/β^-, Lyt-2$^-$, L3T4$^-$ phenotype makes these cells excellent candidates for the equivalent of the human γ-chain-expressing cells.

Although the biological role of Thy-1$^+$ DEC still remains obscure, their resemblance to distinctive members of the T-cell lineage as well as the fact that these cells dwell in a tissue that harbors cells with established immune functions (e.g., antigen presentation, elaboration of cytokines) points to the epidermis as either a site of immature thymocyte migration or, more interestingly, extrathymic T-cell development (45).

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