T-Cell Receptor $\gamma\delta$ Bearing Cells in Normal Human Skin

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T-cell antigen receptors (TCR) are divided into common $\alpha\beta$ and less common $\gamma\delta$ types. In the murine skin, TCR $\gamma\delta^+$ cells have been reported to form the great majority of epidermal T lymphocytes. We have examined the relative contribution of TCR $\alpha\beta^+$ and TCR $\gamma\delta^+$ cells to the T-cell population in normal human skin. Serial sections of freshly frozen skin specimens were acetone fixed, incubated with anti-CD3, βF1 (anti-TCR $\alpha\beta$), anti-TCR $\gamma\delta$-1 and anti-TCR δ1 (anti-TCR $\gamma\delta$) monoclonal antibodies (MoAb), and stained with a highly sensitive method.

Over 90% of the T cells of normal human skin are localized around the postcapillary venules of the dermis, while less than 5% are present within the epidermis. In papillary dermis, TCR $\gamma\delta^+$ cells formed on average 7% (anti-TCR $\gamma\delta$-1) or 9% (anti-TCR δ1) of the total number of CD3$^+$ cells, while TCR $\alpha\beta^+$ cells constituted up to 80%. In epidermis, these percentages were 18% and 29% for TCR $\gamma\delta^+$ cells, and up to 60% for TCR $\alpha\beta^+$ cells.

It is concluded that there is no preferential immigration or in situ expansion of TCR $\gamma\delta^+$ T cells in normal human skin, because the relative percentages found for the TCR $\alpha\beta^+$ and TCR $\gamma\delta^+$ populations in skin are comparable to those found in lymphoid organs and peripheral blood. However, the percentage of TCR $\gamma\delta^+$ cells in epidermis seemed on average higher than in papillary dermis. Therefore, there may still be a difference in migration patterns of TCR $\gamma\delta^+$ v TCR $\alpha\beta^+$ cells, but this does not result in their preferential localization in human epidermis. The hypothesis that TCR $\gamma\delta^+$ T cells have a specialized function in immunosurveillance of epithelia may thus not be valid for human epidermis. *J Invest Dermatol 94:37–42, 1990*

The human integument harbors a wide variety of immune-response-associated cells, forming a complexity recently denominated the skin immune system (SIS) [1]. The epidermal compartment, mainly composed of keratinocytes, contains dendritic Langerhans' cells (LC) and indeterminate cells [2] as well as T lymphocytes [3,4]. Quantitative immunophenotyping studies in vertical sections of normal human skin have revealed that the mean number of CD3$^+$ T cells in the epidermis extrapolated to 10-mm section width is 8.6, of which the majority is CD8$^+$ [4]. However, the great majority of T lymphocytes in human skin is present in the papillary dermis (on average 275 CD3$^+$ cells per 10-mm section width, evenly distributed over the CD4$^+$ and CD8$^+$ subpopulations [4]. These dermal T cells are localized mainly around the postcapillary venules.

Recently, two different types of T lymphocytes have been defined on the basis of expression of either TCR $\alpha\beta$, responsible for recognition of foreign antigens in the context of molecules of the major histocompatibility complex (MHC) [5], or TCR $\gamma\delta$ [6]. Upon stimulation, human TCR $\gamma\delta^+$ cells can display cytolytic activity [7,8] and/or produce lymphokines [9]. The majority of TCR $\gamma\delta^+$ cells described thus far exert MHC non-restricted cytolytic activity, which is lymphokine induced [10]. Also, TCR $\gamma\delta^+$ cells that recognize allogeneic MHC molecules have been described in mouse and man [11–13]. However, the exact role these cells play in the immune system and their natural ligand(s) is not yet defined.

TCR $\gamma\delta^+$ cells constitute on average 5% of T cells in normal peripheral blood and on average 1% of CD3$^+$ thymocytes, with a wide variation between donors [10,14]. The majority of TCR $\gamma\delta^+$ cells are of the CD4-8$^-$ phenotype, but dependent on the donor, significant percentages of CD8$^+$ cells can be found, while CD4$^+$ cells occur in very low frequency [10]. Similar percentages have been reported for TCR $\gamma\delta^+$ cells in murine peripheral lymphoid organs and thymus. These TCR $\gamma\delta^+$ cells are also predominantly CD4-8$^-$; however, in murine epidermis, the great majority of T lymphocytes express TCR $\gamma\delta$ rather than TCR $\alpha\beta$ [15–19]. Such a situation is also encountered in murine intestinal epithelium, where TCR $\gamma\delta^+$ cells of the CD8$^+$ phenotype are the predominant T-cell population [20,21]. These findings have led to the hypothesis that TCR $\gamma\delta^+$ cells preferentially home into epithelia where they play a

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Abbreviations:
- AP: alkaline phosphatase
- APAAP: AP anti-AP complex
- CD: cluster of differentiation
- DE(T)C: dendritic epidermal (T) cells
- LC: Langerhans' cells
- MHC: major histocompatibility complex
- MoAb: monoclonal antibody
- PBS: phosphate buffered saline
- SIS: skin immune system
- TCR: T cell receptor

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role in immunosurveillance against infections and malignant transformation [21–23].

TCR γ and δ genes rearrange at an early stage in thymic differentiation, prior to or coinciding with β, but preceding α gene rearrangement [24–27]. In the mouse, the earliest TCR γ gene rearrangement event involves the Vγ3 gene segment [28,29]. Cells expressing Vγ3 are found in the early fetal thymus but are also the predominant T-cell population in the murine skin. From this observation it has been postulated that this first wave of thymocytes may home into the skin [29]. Alternatively, TCR γ and δ gene rearrangements can also take place extrathymically as evidenced by the occurrence of TCR $\gamma\delta^+$ cells in athymic mice [31–33]. The site(s) where such differentiation takes place is unknown, but it is conceivable that the special epithelial microenvironment of the skin serves such a purpose.

To test whether such hypotheses would be valid for the human SIS, we have quantitated the distribution of TCR $\gamma\delta^+$ and TCR $\alpha\beta^+$ T lymphocytes in a series of skin specimens obtained from 10 normal donors. We have employed two previously characterized MoAbs, reactive with all molecular forms of human TCR $\gamma\delta$ described thus far [10,34], a MoAb specific for TCR $\alpha\beta$ (35), and an anti-CD3 MoAb, to quantitate the relative contribution of TCR $\gamma\delta^+$ and TCR $\alpha\beta^+$ cells to the T-cell population of normal human skin.

MATERIALS AND METHODS

Normal Human Skin Biopsies Biopsies were obtained with informed consent from clinically (confirmed histologically) normal skin of volunteers. A total of 10 biopsies, all from the trunk, were studied. Tissue specimens were frozen in liquid nitrogen stored at −90°C until use.

MoAb Anti-TCR $\gamma\delta$-1 MoAb (hybridoma name 11F2) detects all molecular forms of TCR $\gamma\delta$ described thus far [10]. Anti-TCR δ1 MoAb [34] was purchased from T Cell Sciences (Cambridge, MA) and reacts identically with viable TCR $\gamma\delta^+$ cells as anti-TCR $\gamma\delta$-1 (unpublished observations). Both MoAb can therefore be termed pan-anti-TCR $\gamma\delta$. They were used as purified Ig at 14 μg/ml and 0.3 μg/ml. Expression of TCR $\alpha\beta$ was determined with the MoAb βF1 (1:100 dilution, T Cell Sciences; 35). To study concurrent expression of other glycoproteins by TCR $\gamma\delta^+$ cells of skin, serial sections were stained as described below with the following MoAb: anti-CD3 (1:50 dilution, anti-Leu-4; Becton Dickinson, Mountain View, CA), anti-CD8 (1:50 dilution, anti-Leu-2a; Becton Dickinson), and anti-CD1a (1:150 dilution, OKT6; Ortho Pharmaceuticals, Raritan, NJ).

Immunohistochemistry Different immunohistochemical techniques were compared on cryostat sections of fresh-frozen normal human neonatal thymus, normal human lymph node tissue, and on cytospin preparations of TCR $\gamma\delta^+$ T-cell clones, serving as positive controls. The following method was selected: 6-μm thick vertical skin cryostat sections, fixed with fresh, pure acetone at 4°C on polylysine pre-treated slides, were preincubated in normal goat serum (1:10 dilution, 10 min) and incubated successively with: −1- MoAb diluted as described above (60 min); −2- rabbit anti mouse Ig, (Dakopatts, Glostrup, Denmark) (1:25 dilution, 30 min); −3- alkaline phosphatase (AP) anti-AP complex (APAAP; Dakopatts) (1:50 dilution, 30 min); −4- rabbit anti mouse Ig (20 min); −5- APAAP (20 min). For negative controls, first- and second-step reagents were omitted. Between incubations, sections were rinsed in phosphate-buffered saline (PBS). AP activity was detected histochemically [4]: a carmine red color was developed using naphtol-AS-MX-phosphate (Sigma Chemical Co., St. Louis, MO) as substrate and Fast Red Violet LB (Sigma) as azo dye. Endogenous AP activity was inhibited with 1 mM levanmizole (Sigma) added to the incubation medium. Nuclei were counter-stained by Mayers hematoxylin.

Lymphocyte Enumeration We have determined the total number of CD3+, TCR $\alpha\beta^+$, and TCR $\gamma\delta^+$ lymphocytes within epidermis and papillary dermis as follows: cells were detected with the relevant MoAb and counted in seven different sections of normal human skin specimens from each of the ten donors. For counting, skin sections were divided into suprabasal and intrabasal compartments within the epidermis and into free and perivascular compartments within the papillary dermis. Cell numbers thus obtained were extrapolated to 10-mm section width. Means and ranges of 70 enumerations for each MoAb were determined.

Immunophenotyping of Serial Sections To determine coexpression of different membrane molecules on TCR $\gamma\delta^+$ lymphocytes, seven separate sets of six serial sections of each of ten skin specimens were made. Serial sections were incubated with βF1, OKT6, anti-TCR $\gamma\delta$-1, anti-Leu-4, anti-TCR δ1, and anti-Leu-2a, in this order. In this way, expression of CD1a, CD3, and CD8 could be traced back to TCR$\gamma\delta^+$ cells by comparing adjacent sections.

RESULTS

Enumeration of CD3$^+$, TCR $\alpha\beta^+$, and TCR $\gamma\delta^+$ Cells in Vertical Sections of Normal Human Skin A total of ten different donors were studied. Per donor, four sets of seven different skin sections were made for staining with either anti-CD3, anti-TCR $\gamma\delta$ (two different MoAb), or anti-TCR $\alpha\beta$ MoAb. Only cells that showed plasma membrane staining were counted, because with the technique used, negative control PBS as well as all MoAb gave aspecific, weak, cytoplasmic staining of basal keratinocytes. T cells were enumerated as present in various compartments of human skin thought to reflect different sites for T-cell migration from an immunodynamical point of view [36]. Mean numbers and ranges of positive cells present in the different skin compartments, as well as in epidermis and papillary dermis as a whole, are given in Table I.

The mean number of CD3$^+$ cells detected per 10 mm total skin section width was 267.97 (Table I), which is consistent with the number we have reported previously [4]. The great majority of skin T lymphocytes is located in the papillary dermis, rather than in the epidermis, and particularly at perivascular sites. The reticular dermis also contains a substantial number of T cells, which we have not determined here, but which were 164.05 on average, according to our previous study [4]. Therefore, we can conclude that CD3$^+$ cells present in epidermis form less than 2% of total number of T cells in normal human skin.

Table I. Mean Numbers and Ranges of Total T cells and TCR $\alpha\beta^+$ and TCR $\gamma\delta^+$ Subpopulations Present Per Vertical Section of 10-mm Width at Different Sites Within Normal Human Skin

	Epidermis		Papillary Dermis		
	Suprabasal	Intrabasal	Free	Perivascular	Total
CD3$^+$	0.72	5.71	20.99	247.07	267.97
(anti-Leu4)	0.00–11.72	0.00–52.94	0.00–95.00	23.73–780.00	25.42–881.58
TCR $\alpha\beta^+$	0.36	3.48	11.63	201.90	216.83
(βF1)	0.00–7.02	0.00–27.69	0.00–45.22	62.86–747.69	62.86–815.38
TCR $\gamma\delta^+$	0.10	1.09	2.03	15.96	19.10
(anti-TCR $\gamma\delta$-1)	0.00–4.44	0.00–7.41	0.00–16.17	0.00–111.43	0.00–125.71
TCR $\gamma\delta^+$	0.29	1.56	2.64	20.72	25.15
(TCRδ1)	0.00–11.11	0.00–15.79	0.00–25.64	0.00–133.33	0.00–171.79

Table II. Percentages of CD3+ Lymphocytes in Normal Human Skin Expressing TCR αβ or TCR γδ (CD3+ = 100%)*

	TCR αβ (βF1)	TCR γδ (anti-TCR γδ-1)	TCR γδ (TCRδ1)
Epidermis	60%	18%	29%
Suprabasal	50%	14%	40%
Intrabasal	61%	19%	27%
Papillary Dermis	80%	7%	9%
Free	55%	10%	13%
Perivascular	82%	7%	8%
Total	81%	7%	9%

* Calculated from Table I.

Table II gives the percentages of CD3+ cells expressing TCR αβ or TCR γδ in the various skin compartments as calculated from the numbers given in Table I. Clearly, the great majority of T lymphocytes of normal human skin at whatever location, express TCR αβ rather than TCR γδ. Depending on the MoAb used, either anti-TCR γδ-1 or anti-TCR δ1, the percentages of TCR γδ+ cells in epidermis were 18% or 29%, respectively. Figure 1*a,b* give an impression of the localization and morphology of these TCR γδ expressing cells in the epidermis (intrabasal and suprabasal, respectively). Within the epidermis, TCR γδ+ cells often displayed a slightly dendritic morphology. Within the papillary dermis, TCR γδ+ cells formed 7% or 8% of perivascular T cells and 10% or 13% of less common free T cells (again depending on the MoAb used). At these sites, TCR γδ+ cells always had a typical lymphoid appearance. Although TCR γδ+ cells were never the predominant T lymphocyte type at any location in the skin, the epidermis contained relatively more TCR γδ+ cells than the papillary dermis.

Phenotype of TCR γδ+ Lymphocytes as Determined in Serial Sections Double staining techniques were unsatisfactory to determine coexpression of various antigens with TCR γδ, mainly because of the low frequency, but particularly because of the widely varying number of TCR γδ+ cells present in different sections. In many sections, no TCR γδ+ cells could be detected (see ranges in Table I). Therefore, we could not reliably interpret staining specificity on skin sections, although this was satisfactory on 100% positive TCR γδ+ T-cell clones serving as positive controls.

For these reasons, we have employed the serial section approach. Anti-CD3 staining on TCR γδ+ cells was checked as a specificity control, because the TCR requires CD3 for cell-surface expression and should therefore always be coexpressed with this molecular complex [37]. TCR γδ+ cells, especially when present in small clusters, could be traced back in adjacent sections stained with anti-CD3. We have no evidence that TCR γδ staining occurred in the absence of anti-CD3 staining.

The majority of TCR γδ+ cells present in human peripheral blood have the CD4-8− phenotype, but a varying proportion express CD8, depending on the donor. Murine dendritic epidermal TCR γδ+ are also double negative [19], but intestinal epithelial TCR γδ+ cells have been shown to express predominantly CD8, and have been postulated to constitute a particular subset of TCR γδ+ cells [20,21]. For this reason we were interested in studying coexpression of CD8 with TCR γδ in human skin. Only epidermal T cells were studied because the high frequency of CD8+ TCR αβ+ cells in papillary dermis made interpretation of serial sections impractical.

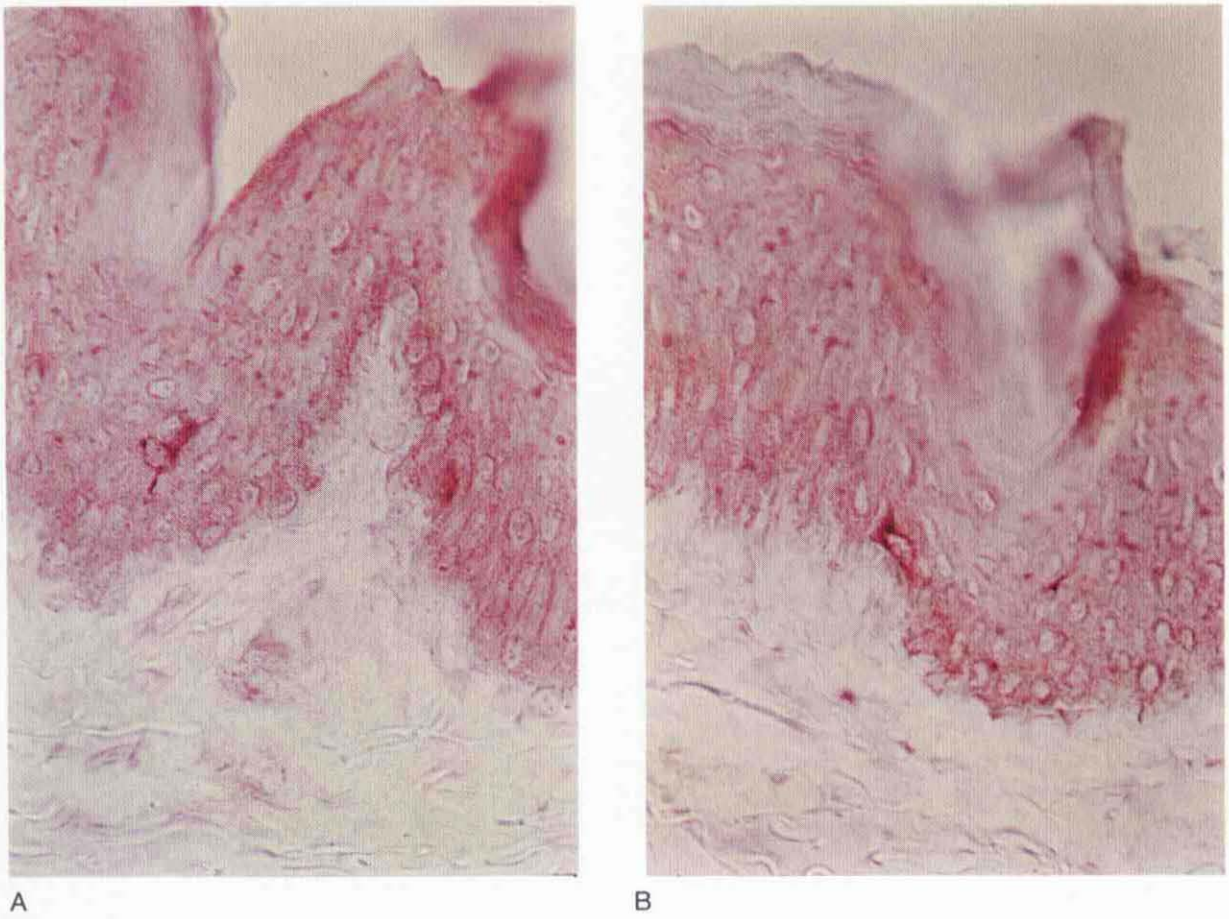

Figure 1. TCR γδ expressing cells in vertical section of normal human skin. These cells were mostly localized intrabasally *(a)* or directly suprabasal *(b)* in the spinal layer of epidermis (original magnification 400 ×).

Within epidermis no $CD8^+$ cells were detected in the same location as anti-TCR $\delta 1^+$ cells in adjacent sections.

In LC enriched epidermal cell suspensions we observed occasional cytoplasmic, but no plasma membrane staining with the two different anti-TCR $\gamma\delta$ MoAb as intact antibodies as well as $F(ab')_2$ fragments (38 and unpublished). Therefore, we were curious to know whether epidermal TCR $\gamma\delta^+$ cells also showed reactivity with anti-CD1a and MoAb in tissue sections. Occasional epidermal cells displaying extensive dendritic morphology stained in serial sections with anti-CD1a as well as with anti-TCR $\gamma\delta$ MoAb. It remains to be determined whether this points towards a relationship between LC and TCR $\gamma\delta^+$ cells or it is a staining artifact.

DISCUSSION

Since 1982, several laboratories have reported the presence of Thy-1^+ dendritic epidermal cells (DEC) in normal mouse epidermis [39–42]. The Thy-1 marker is not T-cell specific, because it is also expressed in mouse brain. However, murine DEC were defined as belonging to the T-cell lineage because they reacted with antibody to the CD3 molecular complex. Next, it was shown that murine DEC predominantly express CD3 in association with the alternative TCR $\gamma\delta$, rather than the common TCR $\alpha\beta$, expressed on most peripheral T cells [15–19]. Because it became apparent that DEC were a special intra-epithelial and dendritic population of T lymphocytes, they have been renamed dendritic epidermal T cells (DETC) [19]. Two extensive searches for the human analogue of murine Thy-1^+ DETC were unsuccessful [3,43], which was perhaps due to the fact that in man the Thy-1 marker is not expressed on bone marrow-derived cells but only in the brain [44]. Recently, MoAb directed against human TCR $\gamma\delta$ have become available. This has made it possible to reinvestigate the existence of a human analogue of DETC. We have stained a number of normal human skin sections obtained from 10 adult donors with these anti-TCR $\gamma\delta$ MoAb.

Within the epidermis, TCR $\gamma\delta^+$ cells formed a minority of intra-epithelial T lymphocytes. Depending on the MoAb used, percentages as expressed in relation to the total mean number of $CD3^+$ cells varied and constituted 18% and 29%, respectively, for anti-TCR $\gamma\delta$-1 and anti-TCR $\delta 1$ (Table II). TCR $\alpha\beta^+$ cells made up 60% of intra-epidermal $CD3^+$ cells. The majority of the intra-epidermal TCR $\gamma\delta^+$ lymphocytes were localized in the basal layer, which does not differ essentially from the localization of TCR $\alpha\beta^+$ cells within the epidermis (Table I). The wide range in numbers of TCR $\gamma\delta^+$ as well as TCR $\alpha\beta^+$ cells as expressed per 10-mm epidermal section width, is in accordance with the observation that intra-epidermal T lymphocytes occur in clusters [45]. In the epidermis, TCR $\gamma\delta^+$ cells often showed a slightly dendritic morphology (Fig 1*a,b*), presumably because they are localized between keratinocytes that are known to have extensive intercellular adhesion structures. Occasionally, TCR $\gamma\delta^+$ cells with highly dendritic morphology were observed, especially within hair follicle epithelium. In serial sections, these rare, highly dendritic TCR $\gamma\delta^+$ cells also expressed CD1a.

In the dermis, TCR $\gamma\delta^+$ cells formed, again depending on the MoAb used, 7% or 9% of the mean number of $CD3^+$ cells counted in adjacent sections. TCR $\alpha\beta^+$ constituted 80% of dermal $CD3^+$ cells (Table II). As expected in view of our previous study of the distribution of $CD3^+$ cells in normal human skin [4], the majority of both TCR $\alpha\beta^+$ and TCR $\gamma\delta^+$ cells were present around the post-capillary venules. The wide range of both subsets at these perivascular sites (Table I) can be explained by the observation that clusters of dermal T cells are found around some but not all post-capillary venules (unpublished). The morphology of dermal TCR $\gamma\delta^+$ T cells was lymphoid, i.e., round small cells with clearly stained plasma membranes.

Two inconsistencies are obvious from Tables I and II. In the first place, there is a discrepancy between the number and percentages of TCR $\gamma\delta^+$ cells detected with the two different MoAb. Anti-TCR $\gamma\delta$-1 and anti-TCR $\delta 1$ are conspicuous when low numbers of lymphocytes are counted, such as in epidermis, but even out when total skin lymphocytes are considered. Second, the sum of TCR $\gamma\delta^+$ and TCR $\alpha\beta^+$ cells did not reach 100% of $CD3^+$ cells. Both anti-TCR $\gamma\delta$ MoAb stain exactly comparable percentage of cells in unseparated preparations of viable lymphocytes and perform identically when tested on viable TCR $\gamma\delta^+$ clones in immunofluorescence (J.J.M. van Dongen and J. Borst, submitted). Therefore, the discrepancies found here may reflect differences in the reactivity of the MoAb on acetone-fixed tissue. The same problem may play a role in the detection of TCR $\alpha\beta^+$ cells with βF1 MoAb.

It is difficult to understand that LC may express TCR $\gamma\delta$, which is suggested by our observations [38] and by those of other investigators [45]. As reported here, highly dendritic epidermal cells, which reacted with anti-TCR $\gamma\delta$ MoAb as well as with anti-CD1a in adjacent sections, have been found in occasional tissue sections. In addition, over 10% of CD1a-positive cells in LC enriched human epidermal cell suspensions showed reactivity with anti-TCR $\gamma\delta$ MoAb. This was observed only in cytospin preparations of acetone fixed, permeabilized cells, but not in suspensions of viable cells, indicating that reactivity occurred with cytoplasmic components. This reactivity was observed with anti-TCR $\gamma\delta$-1 in the form of intact Ig, as well as $F(ab')_2$ fragments, and with anti-TCR $\delta 1$, anti-TiγA [13], and δTCS1 MoAbs [14], while negative controls were satisfactory (M.B.M. Teunissen, unpublished). The possible expression of TCR $\gamma\delta$ in LC is puzzling, particularly in view of the fact that until now, no CD3 expression has been observed on these cells, while it is known that CD3 expression is an obligate requirement for TCR expression [37]. The option that human LC would contain molecules homologous or identical to TCR $\gamma\delta$ (exclusively in the cytoplasm) clearly needs further investigation.

In conclusion, our findings suggest that with respect to their distribution within normal human skin TCR $\gamma\delta^+$ T cells behave analogous to TCR $\alpha\beta^+$ T cells. TCR $\gamma\delta^+$ lymphocytes are mainly localized around the post-capillary venules and constitute about 8% of the total mean number of $CD3^+$ T cells in normal human skin (Table II). This ratio is comparable to that found in peripheral blood and lymphoid organs [10,14,45]. However, within the epidermis, TCR $\gamma\delta^+$ cells formed a slightly higher percentage of total T cells than in the dermis. Therefore, there may be a difference in migration pattern of TCR $\gamma\delta^+$ cells v TCR $\alpha\beta^+$ cells into the epidermis, but this does not result in a predominance of TCR $\gamma\delta^+$ cells at this site.

The situation in adult human skin thus seems to be substantially different from that described for murine skin. A human analogue for the murine DETC has not been identified as such, with regard to numbers, distribution, and morphology. Future studies, especially into the ontogeny of the T cells of SIS, might reveal whether at an earlier point in human development there is a situation comparable to that observed in the mouse. Preliminary studies have not revealed higher percentages of TCR $\gamma\delta^+$ cells in human neonatal skin than in adult skin (T. Vroom et al., in preparation).

Given the cytotoxic potential of TCR $\gamma\delta^+$ cells and their presence in murine epithelia, it has been hypothesized that TCR $\gamma\delta$ expressing cells may play a role in continuous immune surveillance of epithelia [21–23]. If one would solely take into consideration the relative number of human epidermal T cells expressing TCR $\gamma\delta$, their frequency would argue against such a function in man. However, analysis of ligand specificity, proliferative responses to antigenic stimuli and/or lymphokines, and effector functions of TCR $\gamma\delta^+$ epidermal cells will reveal whether both T-cell subsets play similar or supplementary roles in the immunophysiology and immunopathology of SIS.

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