Tissue distribution of human $\gamma\delta$ T cells: No evidence for general epithelial tropism

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Abstract

In man and mice only a small proportion of T cells in the peripheral lymphoid compartment express the $\gamma\delta$ T cell receptor (TCR). In mice, however, $\gamma\delta$ T cells comprise the predominant population at particular epithelial sites—in epidermis and epithelia of intestine, reproductive organs, and tongue. The distribution of $\gamma\delta$ T cells in normal human tissues was investigated, paying particular attention to epithelial layers. In all lymphatic organs and in epithelia of a wide variety of non-lymphatic organs, including the respiratory tract, male and female reproductive organs and tongue, $\gamma\delta$ T cells constituted less than 5% of total T cells, with the remainder expressing TCR $\alpha\beta$. The only exception was the intestine, where $\gamma\delta$ T cells were preferentially situated in the columnar epithelium of the crypts, rather than in the lamina propria.

It is concluded, therefore, that human $\gamma\delta$ T cells do not display a general epithelial tropism and are, in terms of relative numbers, no more able than $\alpha\beta$ T cells to carry out continuous surveillance of the immune system against infection or transformation in epithelia. $\gamma\delta$ T cells may, however, have a specialised function in the epithelium of the intestinal tract.

Two types of T cell antigen receptors (TCRs) are now known, the $\alpha\beta$ and the $\gamma\delta$ heterodimer, which are expressed at the cell surface in association with the CD3 molecular complex.¹⁻³ Each TCR chain contains a variable and a constant domain, encoded by different gene segments V, (D), J and C that combine by rearrangement during T cell differentiation. Although the γ and δ loci together contain fewer different gene segments than the α and β loci, the potential repertoire of TCR $\gamma\delta$ is very large as one δ gene can incorporate more than one D segment,⁴ which, with the addition of nucleotides at the junctions, gives rise to a great variety of sequences.¹ At present, it is not clear what contribution $\gamma\delta$ T lymphocytes make to the immune system. They may complement $\alpha\beta$ T cells in terms of antigenic specificities, functional capabilities, or sites of action within the body.

In mice, $\gamma\delta$ T cells form a minority of the total T cell population in the thymus and

peripheral lymphoid organs.^{5,6} More than 90% of T cells in the murine epidermis, however, express TCR $\gamma\delta$,^{7,8} while $\gamma\delta$ T cells have also been reported to constitute a very large proportion of T cells in the epithelia of reproductive organs,⁹ tongue,⁹ and intestine.¹⁰ Strikingly, certain V γ and V δ gene segments are expressed predominantly in different murine tissues. Moreover, the diversity within the $\gamma\delta$ T cell population differs greatly, depending on the tissue. The epidermal $\gamma\delta$ T cells express V5/J1/C γ 1 and V1/D2/J2/C δ encoded receptor chains¹¹ and can be considered clonal, likewise the $\gamma\delta$ T cell population in reproductive organs and tongue that expresses V6/J1/C γ 1 and V1/D2/J2/C δ encoded receptor chains.^{9,12} In intestinal epithelium^{11,13} and in peripheral lymphoid organs¹⁴ a large TCR $\gamma\delta$ repertoire exists that mainly depends on junctional diversity, while V γ 7 and V γ 4 gene segments are used preferentially at these respective sites. Due to their specific localisation in certain murine epithelia, their limited receptor diversity at two of these sites, and their potential specificity for the evolutionary conserved heat shock proteins,¹⁵ it has been postulated that $\gamma\delta$ T cells may have a specialised role in the continuous immunosurveillance of epithelia.¹⁶⁻¹⁸

In man TCR $\gamma\delta$ bearing cells constitute less than 2% of CD3 positive thymocytes and less than 1–20% of peripheral blood T cells.^{19,20} In lymphoid organs $\gamma\delta$ T cells constitute less than 5% of the T lymphocytes.^{20,21} With respect to the epithelial localisation of human $\gamma\delta$ T cells one clear difference with the murine system is already apparent: in human skin $\gamma\delta$ T cells are not the predominant T cell population, nor in epidermis nor dermis.^{20,22} In intestine the situation seems more comparable between man and mice, with human $\gamma\delta$ T cells localising preferentially in the epithelium rather than in the lamina propria.²³⁻²⁵

We have quantitated $\gamma\delta$ and $\alpha\beta$ T cells in a great variety of normal human lymphatic and non-lymphatic tissues, including the respiratory system, the male and female urogenital tract, and the tongue, which had not previously been investigated. Special attention was paid to the various epithelial layers within these tissues to shed light on the possible function of human $\gamma\delta$ T cells in epithelial surveillance.

Methods

Normal tissues were obtained from necropsies or from normal parts of surgical specimens.

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Samples were derived from adults, unless otherwise indicated. For every type of tissue at least two samples from different subjects were investigated. The following tissues were examined: (1) lymphatic and haemopoietic organs (a) thymus, fetal (12 weeks pregnancy) and neonatal, (b) lymph node, neonatal and adult, (c) tonsil, infantile, (d) spleen, (e) bone marrow, (f) liver, fetal (12 weeks pregnancy), neonatal and adult; (2) skin, fetal and adult; (3) digestive tract (a) tongue, (b) salivary glands, (c) oesophagus, (d) stomach, (e) small intestine, (f) large intestine, (g) appendix; (4) urogenital tract (a) kidney, (b) ureter, (c) urethra, (d) vagina, (e) uterus and cervix, (f) testis and epididymis; (5) respiratory tract (a) nasal cavity, (b) trachea, (c) lung.

Small tissue blocks were snap-frozen and stored in liquid nitrogen. Sections (8 μ m thick) were cut on a Reichert-Jung 2800 Frigocut Cryostat (Reichert-Jung GmbH, Nussloch, Germany), air dried, and fixed in acetone for 10 minutes. Fixation and all subsequent washes and incubations were performed at room temperature. After fixation, sections were washed three times in phosphate buffered saline (PBS), pH 7.2. For the immunoalkaline phosphatase detection method sections were incubated with monoclonal antibody, diluted in PBS with 1% bovine serum albumin (BSA) (PBS/BSA), washed three times in PBS and incubated with alkaline phosphatase conjugated rabbit anti-mouse immunoglobulin (RaMIg-AP, Dakopatts D314, Glostrup, Denmark), diluted 1 in 20 in PBS with 10% normal human serum. For the alkaline phosphatase-anti-alkaline phosphatase detection method, sections were first incubated with undiluted normal rabbit serum, followed by incubation with monoclonal antibody and then unconjugated rabbit anti-mouse immunoglobulin (Dako Z259), diluted 1 in 25 in PBS/BSA with 10% normal human serum, as second step reagent, followed by washing with PBS and incubation with alkaline phosphatase-anti-alkaline phosphatase complex (Dako D651), diluted 1 in 40 in PBS/BSA. Subsequently, sections were washed three times in TRIS-buffered saline, pH 7.6, and incubated with staining solution for 30 minutes in the dark. This solution was made according to the method of Li *et al.*,²⁶ with modifications. It contained 0.5% v/v of a freshly prepared 1:1 mixture of 4% w/v New Fuchsin (Chroma 1B467, Stuttgart, Germany) in 2 M HCl and 4% w/v NaNO₂ in H₂O, 3% v/v of a solution of 1% w/v Naphtol AS-MX phosphate (N-5000, Sigma Chemical Co, St Louis, Missouri) in dimethylformamide and 0.025% w/v Levamisole (Sigma L-9756). The staining solution was made in 0.2 M TRIS-HCl, pH 8.0, and filtered before use. After staining, sections were rinsed with distilled water, counterstained with Mayer's haematoxylin, washed in running tap water and mounted in glycerin-gelatin. Negative control sections were processed as described with omission of the incubation step with monoclonal antibody. Apart from immunohistochemistry, one section of each sample

was stained with haematoxylin and eosin for histological examination.

Monoclonal antibodies used for staining tissues were: Identi-T β F1 (anti-TCR $\alpha\beta$)²⁷ from T cell Sciences, Cambridge, Massachusetts, used at 2 μ g/ml; anti-TCR $\gamma\delta$ -1 (hybridoma name 11F2, anti-TCR $\gamma\delta$),¹⁹ used at 2–4 μ g/ml; CLB-T3 (anti-CD3) from Dr R van Lier, Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands, used at 5 μ g/ml. Staining with anti-TCR monoclonal antibodies was done according to the alkaline phosphatase-anti-alkaline phosphatase method, with other monoclonal antibodies according to the alkaline phosphatase method. Anti-TCR monoclonal antibodies were tested for reactivity on cytocentrifuge preparations of TCR $\alpha\beta$ positive and TCR $\gamma\delta$ positive T cell clones.¹⁹

Results

To determine the proportion of T lymphocytes that expressed either TCR $\alpha\beta$ or TCR $\gamma\delta$, serial sections were stained with anti-TCR $\alpha\beta$, anti-CD3, and anti-TCR $\gamma\delta$ monoclonal antibodies, in this order. Only tissue sections that showed no evidence of inflammation were included, but intestine, lung, and endometrium and vagina always show more or less reactive lesions.

LYMPHATIC AND HAEMOPOIETIC ORGANS

In neonatal and fetal thymus less than 5% of CD3 positive cells expressed TCR $\gamma\delta$. In accordance with published data,²⁰ $\gamma\delta$ T cells were found preferentially in the juxta-medullary region of the cortex and in the medulla and rarely present in the outer cortex. No preferential association of $\gamma\delta$ T cells with Hassall's corpuscles was found.

In lymph nodes, tonsil, and Peyer's patches less than 5% of CD3 positive cells expressed TCR $\gamma\delta$. In contrast to $\alpha\beta$ T cells, $\gamma\delta$ T cells were not found within the lymph follicles. Of the numerous T cells present within the lympho-epithelial area of the tonsillar crypts, less than 5% expressed TCR $\gamma\delta$.

In spleen $\gamma\delta$ T cells were again less than 5% of total CD3 positive cells in the periarteriolar lymphoid sheath outside the lymph follicles and in the sinusoids of the red pulp. We could not confirm the finding of others^{21,25} that $\gamma\delta$ T cells are relatively more abundant in the red pulp than in the periarteriolar sheaths.

In normal bone marrow and fetal (12 week pregnancy), neonatal, and adult liver $\gamma\delta$ T cells constituted less than 5% of total CD3 positive cells.

EPITHELIAL TISSUES

Skin

In agreement with published data^{20,22} $\gamma\delta$ T cells were extremely rare in skin sections (less than 1% of CD3 positive cells) derived from adults, as well as in one fetal specimen (12 week pregnancy). When observed, they were localised in the basal layer of the epidermis or, in higher numbers, perivascularly in the papillary dermis.

Figure 1 Section of human vagina showing one of the very rare TCR $\gamma\delta$ positive cells (arrow) near the basement membrane (immunoalkaline phosphatase-anti-alkaline phosphatase staining).

Urogenital tract

In the urogenital tract we looked at ureter, urethra, kidney, uterus, cervix, vagina, ovary, testis and epididymis. In normal renal sections (eight specimens) no $\gamma\delta$ T cells were found. This was not surprising as CD3 positive cells were also only rarely observed. The same holds true for the ureter and the urethra (two specimens each), testis and epididymis (two specimens each). Tissue sections of uterus, cervix, and vagina were derived from patients that had undergone hysterectomy because of the presence of myomata. Therefore, these samples contained reactive lesions. The endometrial layer, including columnar epithelium and lamina propria (two specimens), and the stratified squamous epithelial layers of cervix and vagina (five and two specimens respectively) included normal regions that contained only very few CD3 positive cells, of which none expressed TCR $\gamma\delta$. In slightly inflamed regions the number of CD3 positive cells was increased, both in epithelium and in lamina propria. In these cases, $\gamma\delta$ T cells also constituted less than 5% of total T cells. When present in the squamous epithelium sections they were found in the basal layer (fig 1).

Figure 2 Section of human oesophagus showing one TCR $\gamma\delta$ positive cell near the basement membrane (immunoalkaline phosphatase-anti-alkaline phosphatase staining).

Digestive tract

We paid attention to the tongue, salivary glands, oesophagus, stomach, small and large intestine and appendix. Sections from one tongue sample showed some CD3 positive cells within the squamous epithelium and in the subepithelial layer. None of these T cells expressed TCR $\gamma\delta$. Non-inflamed salivary glands (six specimens) did not contain any T lymphocytes within the epithelium of the ducts and acini. Within the surrounding collagenous stroma, scattered CD3 positive lymphocytes were observed, of which none expressed TCR $\gamma\delta$. In the squamous epithelium of the oesophagus near the basement membrane, occasional $\gamma\delta$ T cells were found, which constituted less than 5% of CD3 positive cells at this site. Figure 2 shows one such intraepithelial $\gamma\delta$ T cell. In inflamed oesophagus most CD3 positive cells also expressed TCR $\alpha\beta$ (more than 95%). In the stomach (three specimens) $\alpha\beta$ T cells were detected in the lamina propria, while in the crypt epithelium $\alpha\beta$ T cells and $\gamma\delta$ T cells could not be positively identified, because of the overwhelming endogenous alkaline phosphatase activity of the epithelial cells. This activity could not sufficiently be inhibited by Levamisole.

Serial sections were prepared from human small and large intestine at different sites (duodenum, jejunum, ileum, sigmoid, cecum, appendix), using tissue samples from six adults. The results can be summarised as follows: quantitated as total cells per relevant surface area, $\alpha\beta$ T cells were localised predominantly in the lamina propria, rather than in the columnar epithelium of the intestinal crypts, while $\gamma\delta$ T cells were localised in the epithelium, rather than in the lamina propria (figs 3A–C). Despite the preferential localisation of $\gamma\delta$ T cells within the intestinal epithelium the absolute number of $\alpha\beta$ T cells was such that they outnumbered $\gamma\delta$ T cells, not only in the lamina propria (more than 95% of CD3 positive cells), but also in the epithelium (70–90% of CD3 positive cells). The relative number of $\gamma\delta$ T cells within the epithelium varied greatly, not only between different sites in the intestinal tract, but also between sections of the same site originating from different subjects. As in the reproductive tract, inflammatory activity may significantly influence the distribution of these two T cell subsets within the intestine.

Respiratory tract

Lung and trachea were difficult to investigate, because all specimens (more than four) showed more or less inflammatory activity. It was clear that, inflamed or not, only a small proportion of CD3 positive cells found in the epithelial layer and in the lamina propria were TCR $\gamma\delta$ positive (less than 5%) (fig 4). In one specimen, however, up to 10% of the T cells were TCR $\gamma\delta$ positive. These were localised primarily in the interalveolar septa. Although in the nasal cavity (eight specimens), CD3 positive cells were found, also intraepithelially, $\gamma\delta$ T cells were observed extremely rarely and then in the lamina propria.

Figure 3 Serial sections of human appendix showing TCR $\gamma\delta$ positive cells (A) CD3 positive cells, (B) TCR $\alpha\beta$ positive cells (C) TCR $\alpha\beta$ positive cells are abundant around a lymph follicle (thick arrows), and found within the lamina propria, but relatively less predominant in the epithelium, while TCR $\gamma\delta$ positive cells are predominantly located within the crypt epithelium (arrow heads and thin arrow). Two cells detected in the sections stained for TCR $\gamma\delta$ as well as for CD3 are indicated by arrow heads (immunoalkaline phosphatase-anti-alkaline phosphatase staining).

Figure 4 Section of human lung showing one TCR $\gamma\delta$ positive cell (arrow) lying in an interalveolar septum (immunoalkaline phosphatase-anti-alkaline phosphatase staining).

Discussion

In the past few years $\gamma\delta$ T cells have been identified in man, mice, chickens, rats, cattle, sheep and pigs. While in man, mice, and rats²⁸ the contribution of $\gamma\delta$ T cells to the total circulating T cell population is low (about 5% on average), the $\gamma\delta$ T cell pool in peripheral blood is relatively large in chickens (25–30% of total T cells,²⁹ sheep,³⁰ cattle³¹ and pigs³² (15–50%, depending on the animal and its age).

In the human thymus $\gamma\delta$ T cells were found in the juxta-medullary region of the cortex and in the medulla, rather than in the outer cortex, in agreement with published data.^{20,21} In the chicken thymus³³ $\gamma\delta$ T cells are also relatively sparse in the cortex, despite their abundance in the periphery. In sheep two phenotypically distinct types of $\gamma\delta$ T cells can be detected that occur in the cortex and in the medulla, respectively.³⁰ The developmental relation between these cell types is unclear. Association with the epithelial cells of Hassall's corpuscles has been reported for bovine³¹ and ovine³⁰ TCR $\gamma\delta$ positive thymocytes, but not for murine cells. We have no evidence for such an association in the human thymus. It remains to be established to what extent $\gamma\delta$ T cells undergo the same thymic differentiation and selection processes as $\alpha\beta$ T cells.

In lymph node, tonsil, Peyer's patches and spleen $\gamma\delta$ T cells constituted a small proportion of total T cells and essentially followed the distribution of $\alpha\beta$ T cells. Strikingly, $\gamma\delta$ T cells were not found at all within lymph follicles. This could be a reflection of their relatively low numbers. They were also not seen in reactive nodes, with high numbers of intrafollicular CD4 positive $\alpha\beta$ T cells. In cattle, where circulating $\gamma\delta$ T cells are much more numerous, intrafollicular $\gamma\delta$ T cells are also rarely observed.³¹ We were not able to substantiate the differential distribution of $\alpha\beta$ and $\gamma\delta$ T cells reported for human^{20,21} and chicken³³ spleen, with $\gamma\delta$ T cells primarily located in the sinusoids and $\alpha\beta$ T cells in the periarteriolar sheath. It remains to be established whether $\gamma\delta$ T cell recirculation differs essentially from $\alpha\beta$ T cell trafficking.

In contrast to the situation in murine epidermis, in the human epidermis $\gamma\delta$ T cells are a

minority of total T cells.^{20,22} The absolute numbers of epidermal T cells per unit of surface area is also significantly lower than in mice,²² while the morphology of the few detectable $\gamma\delta$ T cells is not comparable with that of the highly dendritic murine epidermal $\gamma\delta$ T cells.^{20,22} In chickens epidermal CD3 positive cells also very rarely express TCR $\gamma\delta$.³³ In cattle, $\gamma\delta$ T cells have been reported to occur in high numbers in the skin,³¹ but they are localised primarily in the dermis, reflecting most likely the abundance of $\gamma\delta$ T cells in circulation.³¹

Murine $\gamma\delta$ T cells would also constitute a major proportion of T cells in epithelia of reproductive organs and tongue, according to immunohistology and PCR analysis.^{9,12} The intraepithelial $\gamma\delta$ T cell subsets in murine skin³⁴ and reproductive organs/tongue³⁵ are both derived from the thymus during ontogeny and express monomorphic receptors. In the human female and male reproductive organs and in the tongue, the equivalent of the murine intraepithelial $\gamma\delta$ T cell type could not be found.

Although $\gamma\delta$ T cells have been reported to occur in the murine lung,³⁶ they constitute only about 10% of total intraepithelial T cells. The analysis of human lung tissue gave a similar result.

In our studies the epithelium of human small and large intestine (including appendix) contained more $\gamma\delta$ T cells than any of the other epithelia investigated (10–30% of total T cells). In other parts of the gastrointestinal tract the contribution of $\gamma\delta$ T cells to the intraepithelial T cell population was smaller. In accordance with published results,^{23–25} $\gamma\delta$ T cells were found preferentially within the epithelium of the intestinal crypts, rather than in the lamina propria, where significant numbers of $\alpha\beta$ T cells were present. The same differential distribution of $\alpha\beta$ and $\gamma\delta$ T cells has been reported for the chicken intestine.³³ In sheep and cattle $\gamma\delta$ T cells occur in lamina propria as well as epithelium.^{31,32}

While it cannot be denied that $\gamma\delta$ T cells in various species show certain preferential associations with epithelial cells, we conclude that there is no evidence for general epithelial tropism of $\gamma\delta$ T cells in man or other species. Only within the large and small intestine do $\gamma\delta$ T cells clearly show a preferential intraepithelial localisation in all species investigated. The combined data suggest that the intestinal epithelium harbours a $\gamma\delta$ T cell population that has at least in part developed extrathymically.^{37–39} The relative contribution of $\gamma\delta$ T cells to the total pool of murine intestinal intraepithelial T cells varies significantly, depending on antigenic stimulation. Exposure to antigens leads to an increase of intraepithelial $\alpha\beta$ T cells, while the absolute number of $\gamma\delta$ T cells remains quite constant.³⁸ Most intraepithelial $\gamma\delta$ T cells constitute a resident population that is not directly connected to the circulating pool, while $\alpha\beta$ T cells can enter the epithelium from circulation during infection. In man this is also evidenced by a difference in $\gamma\delta$ T cell repertoire. While in most people the V δ 2 gene segment is expressed preferentially

on peripheral $\gamma\delta$ T cells, the intestinal epithelium harbours predominantly V δ 1 positive $\gamma\delta$ T cells.²⁴ The same sort of discrepancy in V gene usage is observed in mice. In cattle two types of intestinal intraepithelial $\gamma\delta$ T cells can be observed, a resident intraepithelial population (T19 negative) and a $\gamma\delta$ T cell population (T19 positive) in the lamina propria that is most likely derived directly from the large circulating pool of $\gamma\delta$ T cells.³¹ It will be very interesting to examine the specific contribution of this resident intestinal epithelial $\gamma\delta$ T cell population to the immune response. A clue may come from the observed expansion of intestinal $\gamma\delta$ T cells in coeliac disease.²⁵

In other human epithelia $\alpha\beta$ T cells would be the population most fit to perform the function of epithelial surveillance, at least as judged by the relative numbers of cells that could respond to an infectious agent. Obviously, it remains an intriguing question whether $\gamma\delta$ T cells would be more fit to respond to certain antigens because of a more suitable receptor repertoire. Alternatively, $\gamma\delta$ T cells may contribute in the immune response by carrying out a specific function.

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