Massive, sustained γδ T cell migration from the bovine skin in vivo

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Abstract: In all species studied so far, γδ T cells are abundantly present in epithelia. The functions of these cells are largely unknown. Using a lymph duct cannulation method, which is only possible in large animals such as cattle, we show that large numbers of γδ T cells, but not αβ T cells, are constitutively present in pseudoafferent lymph draining bovine skin. The γδ T cells, which are present in pseudoafferent lymph, use Vγ segments that are characteristic for bovine dermal γδ T cells, suggesting that these cells migrated from the skin. Further supporting the origin of these cells is the fact that fluorescent latex beads injected in the skin could be recovered in cells in the pseudoafferent lymph. The cannulation method is minimally invasive, and the lymph flow, which was sustained and remained essentially unaltered during 14 days, closely represents the in vivo situation. The γδ T cells could not be induced to produce IFN-γ, TNF-α, and IL-10, and they did not express co-stimulatory molecules, IL-2 receptor, and MHC Class II molecules. The level of γδ T cell egress was 6.7×10^3 γδ T cells per cm^2 skin per hour, which is enough to deplete all γδ T cells from the skin within 46 h. As this massive γδ T cell migration was observed during 14 days, constant replenishment of these cells must take place. Our data suggest that γδ T cells in tissues fulfill more than exclusively local functions. *J. Leukoc. Biol.* 81: 968–973; 2007.

Key Words: *trafficking · other animals · T lymphocytes*

INTRODUCTION

γδ T cell subsets show a tissue-specific distribution. In humans and mice, this is illustrated by the tissue-specific distribution of TCR γ- and δ-chain variable segments. Human Vδ1-positive T cells are found in intestinal tissue and Vδ2, mainly in blood. Mice have a skin-associated γδ T cell population [also called dendritic epidermal T cells (DETC)], expressing a limited TCR repertoire using Vγ5 and Vδ1 [1] and a Vγ6/Vδ1 population in the reproductive tract and tongue. The physiological functions of the different γδ T cell subsets are not known, but their selective tissue localizations are thought to reflect differences in function. It is generally assumed that tissue-specific γδ T cells expand and function locally. Mouse DETC are thought to be stationary, as skin grafts are populated very inefficiently with recipient DETC, and recipient Langerhans cells do repopulate the skin graft within days [2]. Skin γδ T cells are known to protect against cutaneous malignancies in mice [3], and the proposed mechanism does not involve trafficking of the cells. Recently, human and bovine γδ T cells isolated from tonsil and blood, capable of performing professional APC functions, have been described [4, 5]. One fundamental question to be answered to get more insight into the way specific γδ T cell subsets function is whether they are stationary or trafficking.

In ruminants, γδ T cells are present in relatively high percentages. The γδ T cell population in bovine skin has been well described [6]. Bovine skin γδ T cells use predominantly Vγ3 and Vγ7, small numbers of N-nucleotides, Jγ5, and Cγ5. All skin-derived Vδ sequences belong to the big ruminant Vδ1 family, but there is no dominating segment.

In cattle (*Bos taurus*), it is possible to cannulate lymph ducts draining the skin. Pseudoafferent lymph ducts, which form spontaneously by reanastomosis after surgical removal of a lymph node, are big enough to insert a cannule. Pseudoafferent lymph is indistinguishable from afferent lymph in cellular content, activation status of cells, and cytokine content. We found that γδ T cells are the predominant cells in pseudoafferent lymph draining the skin.

MATERIALS AND METHODS

Animals and surgical procedures

Four Holstein-Frisian calves of different ages were used in this study (see Table 1). The technique of prescapular lymph duct cannulation for collecting pseudoafferent lymph from skin has been reported previously [7]. Bilateral lymphadenectomy of the prescapular lymph node was performed under full anesthesia. Premedication consisted of i.v. application of 1.5 mg medetomidine (Domitor™, Pfizer Animal Health, Germany), 7.5 mg nalbufine (Nubain®, Bristol-Meyers Squibb, New York, NY, USA), 50 mg flunixine-meglumine (Bedozane™, Eurovet, The Netherlands), and 2 g sodium-ampicillin (Ampi-

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dry 5000™, Dopharma, The Netherlands). Anesthesia was induced with propofol (Rapinovet™, Schering-Plough, Kenilworth, NJ, USA; 2–4 mg/kg i.v., dosed to effect). Animals were placed in lateral recumbancy, intubated, and mechanically ventilated. Surgical stage anesthesia was maintained with 1–2% isoflurane (IsoFlo, Abbott Laboratories Ltd., Abbott Park, IL, USA), administered in an oxygen:air (1:1) mixture. During the postsurgical period, animals were treated for 3 consecutive days with ampicillin trihydrate (Praxavet Ampi, 7.5 mg/kg i.m.) and 1 day with flunixine meglumide (Bedozane, 1 mg/kg i.v.). The afferent and efferent lymphatic vessels were allowed to reanastomose in 6 weeks. For bilateral cannulation of the pseudoafferent lymph vessels, animals were anesthesized and treated as described above. A skin incision was made ventral from the old scar and parallel to the jugular groove. Lymph vessels were ligated as distally as possible, and a siliconized and heparinized, kink-resistant PVC cannule (Portex, UK) was inserted 2–3 cm into the lymph vessel, fixated, and externalized through a small skin incision below the surgical incision. Calves were mounted with a neck-shoulder belt with a pocket containing a sterile 200-ml collection flask containing 5 ml PBS, supplemented with 50 IU/ml penicillin and 50 mg/ml streptomycin, which was replaced three times per day. Once daily, each calf was injected with 5000 IU heparin s.c. near the position where the prescapular lymph node was located. Animals were able to move freely in a straw-bedded, 6-m^2 pen with ad libitum access to silage and water. The Animal Committee of the University Utrecht (The Netherlands) approved the experiments that were carried out in The Netherlands, and the experiments that were carried out in the United Kingdom were performed in accordance with the Animal Scientific Procedures Act (1986).

PCR

Pseudoafferent lymph cells were used without further purification. PBMC were isolated from blood by standard Ficoll-Hypaque gradient centrifugation. Cells (2×10^6) were used to isolate RNA with the Qiagen RNAEasy kit (Qiagen, Valencia, CA, USA), followed by first-strand cDNA synthesis with Multiscribe RT. All PCRs were performed in a 20-μl reaction volume containing 5 pmol each primer using PFU polymerase according to the protocol supplied by the manufacturer. The absence of contamination of the cDNA with genomic DNA was confirmed using intron-spanning β-actin primers (β-actin forward: CACCACACCTTTTACAACGAGCTG; β-actin reverse: CTGATCCACATCTGCTGGAAGG) under the following cycling conditions: an initial denaturation of 7 min at 95°C, followed by 30 cycles of 30 s at 95°C, 45 s at 55°C, 1 min at 72°C, followed by a final elongation step of 7 min at 72°C. This β-actin PCR gives a band of 800 bp using pure cDNA and an additional band of 1250 bp when genomic DNA is present.

As the cDNA we used was obtained from different sources (blood and pseudoafferent lymph), we determined the amount of template cDNA that was needed to obtain comparable amounts of Cγ segment PCR product under nonsaturating conditions. This enabled us to adjust the amount of template cDNA used for PCR analysis of Vγ segment use so that each PCR reaction contained comparable amounts of Cγ message. For the Cγ PCR, we used universal primers that amplify all six known bovine Cγ segments (Cγ forward, located at the beginning of the Cγ segment, for quantification: CCCAAGCCCACTRTKTTTCTTCCTTC; Cγ reverse, located at the end of the Cγ part, used for quantification: GTYAGCCAGCTGAACTTCATGTATGTGTC) under the following cycling conditions: an initial denaturation of 7 min at 95°C, followed by 30 cycles of 30 s at 95°C, 45 s at 61°C, 1 min at 72°C, followed by a final elongation step of 7 min at 72°C.

Primers for Vγ segment use analysis (nomenclature according to Herzig et al. [8]): TRGV1, ATGTTGTGGGCCCTAGTGCTGC; TRGV2, GATGATGCGGGTCCCAGCG; TRGV3, ATGTCACCATTGGAAGCATTCACATTTTTC; TRGV4, GCCRTTGTGCACTGGTATCAAGAG; TRGV5, ATGCGGGCCCCAGCACTG; TRGV6, ATGGGCTCTTCTCTCGGGGCA; TRGV7, ATGGCATTCCTGGAAGCGGTCC; TRGV8, ACAAGTTGTCAGTCATGAGGGCTAC; Cγ reverse, a primer that amplifies all six known bovine Cγ segments located at the beginning of the Cγ segment (GAAGGAAGAAAMAYAGTGGGCTTGGG), was used under the following cycling conditions: an initial denaturation of 7 min at 95°C, followed by 35 cycles of 30 s at 95°C, 45 s at 53°C, 1 min at 72°C, followed by a final elongation step of 7 min at 72°C.

Antibodies and flow cytometry

Unlabeled primary mouse mAb against bovine cell surface markers or cytokines that were used in this study include: anti-CD14 (MM61A), anti-workshop cluster 1 (WC1; IL-A29), anti-γδ TCR (N24, GB21A1), anti-CD21 (gb25a), anti-IL-2 receptor (IL-2R) CD25 (CACT108A), and anti-CD3 (MM1a) from Veterinary Medical Research & Development (Pullman, WA, USA); granulocyte marker (IL-A161), kindly provided by Jan Naessens (International Livestock Institute, Nairobi, Kenya); anti-CD1b (CC40), anti-CD86 (N32/52-3), and anti-CD40 (IL-A190), kindly provided by Chris J. Howard and Jane C. Hope (Institute for Animal Health, Compton, UK); anti-MyD-1 (CC149) and anti-NKp46 CD335 (MCA2365) from Serotec (UK); and anti-CD11b (CC94), anti-MHC Class I (IL-A88), and anti-MHC Class II (CC158). For intracellular cytokine stainings, we used the following unlabeled antibodies: anti-TNF-α (49.10, IgG2a) from Cytokine Centre (Utrecht University); anti-TNF-α (6.09, IgG1), kindly provided by Jean-Pierre Scheerlinck (Centre for Animal Biotechnology, University of Melbourne, Parkville, Australia); and anti-IL-10 (MCA2111B) from Serotec. The following conjugated antibodies were used: anti-IFN-γ-FITC (MCA1783) from Serotec; anti-WC1 (IL-A29)-FITC, anti-CD4 (IL-A11)-FITC, anti-CD8 (CC63)-PE, and anti-CD2 (IL-A42)-biotin. As isotype controls, the following antibodies that recognize nonrelevant markers in different species were used: IgG1 (AV20), IgG2b (AV29), and IgG2a (AV37), kindly provided by C. J. Howard and J. C. Hope. The following were secondary antibodies that were used: goat antimouse-PE from BD PharMingen (San Diego, CA, USA); goat antimouse-A633 and streptavidin-A633 from Molecular Probes (Eugene, OR, USA); and antimouse IgG1-FITC, antimouse IgG2a-FITC, and antimouse IgG2b-PE from Southern Biotech (Birmingham, AL, USA). Red fluorescent beads (1 μm) were obtained from Molecular Probes. Before intracellular cytokine stainings, cells were treated for 6 h with 10 μg/ml Brefeldin A (Sigma Chemical Co., St. Louis, MO, USA) in the absence of stimulation or with 1 μg/ml ionomycin (Sigma Chemical Co.), 50 ng/ml PMA (Sigma Chemical Co.) with an additional 10 μg/ml Brefeldin A during the last 6 h of culture, or with 2.5 μg/ml Con A (Sigma Chemical Co.) with an additional 10 μg/ml Brefeldin A during the last 6 h of culture. Stainings for cell surface markers were performed prior to incubation with cytofix/cytoperm solution (BD Biosciences, San Jose, CA, USA). Antibodies against cytokines were diluted in perm/wash solution (BD Biosciences).

RESULTS

Pseudoafferent lymph contains mainly γδ T cells

Flow cytometric analysis of pseudoafferent lymph cells of four calves showed the presence of a large proportion of γδ T cells. Data collected from these animals are summarized in **Table 1**. A representative analysis is shown in **Figure 1a**, where γδ T cells form 93% of the CD3+ population and 63% of the total leukocyte population. Paired blood and pseudoafferent lymph samples, which were collected from two animals, showed that the percentage of γδ T cells was higher in pseudoafferent lymph than in PBMC (Table 1).

An extensive analysis in time was performed on a 3-month-old calf from which pseudoafferent lymph was collected and analyzed three times per day during a period of 14 days (**Fig. 2**). The lymph flow was 20 (4) ml/hour, containing $1.6\ (0.4) \times 10^6$ cells/ml on the average (SD in parentheses) during the 14-day period. The lineage of the other cells present in pseudoafferent lymph was assigned based on expression of the lineage-specific markers CD14 (monocytes), MyD1 [dendritic cells (DC) and granulocytes], CD21 (B cells), CD11b (monocytes and granulocytes), IL-A161 (granulocytes), and CD3 (which allowed us to calculate the percentage of αβ T cells by subtracting the percentage of γδ T cells). Average percentages of cell populations that were found are: 67% (6.4) γδ T cells, 7.1% (2.4) DC, 5.5% (3.7) granulocytes, 6.1% (3.5) monocytes, 7.2% (2.9) αβ T cells, 3.0% (1.1) B cells, and 3.3% (1.6) NK cells.

Phenotype of the γδ T cells

Human Vδ2+ γδ T cells have been shown to express MHC Class II, IL-2R CD25, and the costimulatory molecules CD40

TABLE 1. Animals and Phenotype of γδT Cells in Pseudoafferent Lymph and Blood

Animal	Age (months)	Sex	Cell source	% γδT cells[a] in the total CD3+ population	% γδT cells[a] in the total leukocyte population	% WCI+[a] in the total γδT cell population
001438	3	m	blood	55 (17)	25 (1.0)	65 (5.9)
			ps lymph	93 (10)	67 (6.4)	59 (4.3)
702168	9	m	blood	69	42	57
			ps lymph	78	67	62
401668	9	m	ps lymph	60 (9)	41 (1.7)	62 (0.3)
401626	9	f	ps lymph	55	47	48

[a] Standard deviations are given in parentheses. [b] Number of blood or pseudoafferent lymph samples that were independently collected at different time points and analyzed. [c] CD6 expression was studied in only one blood sample and one pseudoafferent lymph sample of this animal.

and CD86 when they are activated [4]. Like humans, bovine γδ T cells are known to be able to express MHC Class II upon activation [5]. Low levels of IL-2R CD25 are constitutively present on bovine γδ T cells but can be up-regulated upon activation [9, 10]. WC1 is a marker that is specific for ruminant γδ T cell subsets. The two main circulating, ruminant γδ T cell populations are: WC1+/CD2–/CD6–/CD8– and WC1–/CD2+/CD6+/CD8+ [11]. The bovine γδ T cells in pseudoafferent lymph were uniformly CD1b–, CD2–, CD3+, CD4–, CD8–, CD25–, CD40–, CD86–, MHC Class I+, and MHC Class II–, mostly CD6– and partly WC1+ (Fig. 1d and Table 1). The percentages of WC1+ cells in the γδ T cell populations in pseudoafferent lymph are comparable with the percentages in paired blood samples (Table 1) and fall within the normal range [12, 13].

Collected lymph was draining the skin

At the end of the cannulation, fluorescent latex beads were injected s.c. into the skin area that normally drains the prescapular lymph node to check whether the lymph that had been collected and analyzed was indeed draining the skin. Most of the beads appeared associated with cells in pseudoafferent lymph, which was collected 0–8 h after injection (**Fig. 3a**), and almost no beads were recovered 9–16 h after injection, confirming that the cannulated pseudoafferent lymph duct was indeed draining the skin.

Vγ segment use of γδ T cells in two sets of paired pseudoafferent lymph and blood samples was determined by PCR using a primer set that covers all bovine Vγ segments (Fig. 3b). The amount of template cDNA from pseudoafferent lymph and blood was adjusted to contain comparable amounts of Cγ message as described in Materials and Methods. From cDNA from PBMC, all Vγ segments could be amplified, except for Vγ4 (nomenclature as described by Herzig et al. [8] and approved by the Immunogenetics (IMGT) Nomenclature Committee). From pseudoafferent lymph, Vγ1, Vγ3, and Vγ7 could be amplified. The latter two segments have been shown to be the predominant segments in bovine skin [6].

Cytokine expression by pseudoafferent lymph cells

To get an indication of the possible functions of the migratory γδ T cells, we performed intracellular cytokine stainings in combination with stainings for cell surface markers. Subpopulations of bovine γδ T cells have been shown to be able to express IFN-γ [10, 13], which is indicative of a proinflammatory function. TNF-α is an acute-phase inflammatory cytokine, which is produced mostly by macrophages and monocytes, but human skin γδ T cells have been shown to be able to secrete TNF-α in vitro upon TCR triggering [14]. IL-10 is an important immunoregulatory cytokine, which is produced by monocytes and has been shown to be transcribed at low levels by bovine γδ T cells [13]. Stimulation for various amounts of time between 0 and 24 h with ionomycin and PMA or with Con A, followed by addition of Brefeldin A for 6 h, resulted in IFN-γ production by a small subpopulation of CD4+ and CD8+ αβ T cells present in PBMC and by NK cells present in pseudoafferent lymph (**Fig. 4a**). We found no evidence of IFN-γ production by the total γδ T cell population or by the WC1+ subpopulation, regardless of stimulation (Fig. 4b). TNF-α and IL-10 were produced by CD14+ monocytes only and not by γδ T cells or the WC1+ subpopulation (Fig. 4, c–f) under any of the stimulatory conditions tested.

Estimation of γδ T cell traffic

In 1997, Hein and Dudler [6] described γδ T cells in bovine dermis. Using immunohistochemical techniques, they detected 3.1×10^5 γδ T cells per cm^2. This number is higher than the number of murine DETC, which is in the range of 3.1×10^4–5.8×10^4 γδ T cells per cm^2 [15–17]. Pseudoafferent lymph, which was from Animal 001438, contained 1.6×10^6 cells/ml, and the flow per cannule was 20 ml/hour. The prescapular lymph node receives afferents from the skin of the neck, the shoulder, part of the ventral and lateral surfaces of the thorax, and the thoracic limb [18]. We measured the surface of the skin drained by four prescapular lymph nodes of two age-matched animals postmortem, which was 3178 (285) cm^2. From these data, we calculated that 1.0×10^4 cells, including 6.7×10^3 γδ T cells, traffic from 1 cm^2 skin/hour. Without any replenishment, the skin would be emptied of γδ T cells in ~46 h.

DISCUSSION

Heavy trafficking of bovine skin γδ T cells was described in the present study. Our data show that pseudoafferent lymph draining the bovine skin contains a high percentage of γδ T cells. The Vγ segment use of the γδ T cells present in pseudoafferent lymph closely resembles the γδ T cell popula-

TABLE 1. (Continued)

Animal	% CD2+ cells[a] in the total γδT cell population	% CD6+ cells[a] in the total γδT cell population	Samples analyzed[b]	Duration of cannulation and day of sampling
001438	14 (4.3)	13.6[c]	3	
	0	0.9[c]	84	14 days; 3 samples each day
702168	7.3	5.6	1	
	0.8	2.9	1	8 days; day 8
401668	5.5 (2.2)	7.7 (2.1)	3	27 days; day 8, 24, 26
401626	2.1	7.8	1	5 days; day 4

tion in skin, which was described by others [6]. We conclude that there is massive, constitutive efflux of γδ T cells from bovine skin. Our findings are in contrast with the general assumption that epithelial γδ T cells expand and function locally.

It is unknown whether bovine γδ T cells normally extravasate and migrate into the lymph node or pass them quickly on their way to the blood. Low percentages of γδ T cells (1.6% of γδ T cells vs. 40% of CD4+ αβ T cells [19]) have been reported in bovine lymph nodes, including the prescapular lymph node. This finding was consistent in lymph nodes of young calves and adult animals [20]. The few γδ T cells, which were found, were localized mainly in the subcapsular area and the trabeculae and not in the cortex and paracortex, the main B and T cell areas [19, 21, 22]. Recently, it has been shown that bovine WC1+ γδ T cells do not express the lymph node homing marker CCR7 [23]. Together, these data suggest that bovine γδ T cells pass quickly through the lymph nodes and do not migrate into the lymph nodes, appear in the efferent lymphatic ducts, and are transported to the blood via the thoracic duct and the vena cava.

In one animal, the γδ T cell flow was followed for 14 days, and we calculated that without replenishment, the γδ T cell flow could only last for ~46 h. The source of replenishment is unknown but may consist of local proliferation in the skin and/or immigration of cells from the blood. There is evidence for local proliferation of murine DETC [15], estimating that 10% of the cells enter S-phase per 24 h, but this level of proliferation would not be enough to support the massive migration from bovine skin that we report here. Mouse skin grafts are inefficiently populated with recipient DETC [2]. The trafficking patterns of sheep αβ and γδ T cells from blood have been studied before [24], and it was shown that labeled, reinfused, blood-derived γδ T cells stay in the blood, and αβ T cells disappear from the blood, presumably into the lymph nodes. However, the bovine γδ T cells, which we study here, originate from the skin, and it is possible that they recirculate via the blood back to the skin.

It is mostly thought that tissue-resident γδ T cells form a local system forming the first line of defense against developing tumors and invading pathogens. The mechanism of this local defense function is that nonimmune tissue cells, such as keratinocytes or enterocytes, can up-regulate molecules such as MICA (in humans) or Rae-1 (in mice), which trigger nearby γδ T cells to kill [3, 25]. Our data do not argue against a local function of γδ T cells but suggest that they perform additional functions for which trafficking is needed. Antigen-presenting functions by γδ T cells, including bovine γδ T cells, have been reported recently [4, 5], and the migratory capacity of γδ T cells, which we show here, is compatible with APC functions. However, other characteristics of the γδ T cells in pseudoafferent lymph are not consistent with APC functions: Typically, APCs that leave healthy skin are fewer in number and are less-efficient APCs than APCs coming from inflamed tissue [26]; our data are collected in a steady-state situation reflecting homeostasis in healthy tissue. Lymph node homing and APC functions of human blood-derived γδ T cells have to be induced by TCR triggering [14, 27]; the migrating γδ T cells we describe do not express markers that are indicative of recent activation, such as MHC Class II and IL-2R CD25, which are up-regulated upon activation of bovine γδ T cells [5, 10]. The costimulatory molecules CD40 and CD86, which are up-regu-

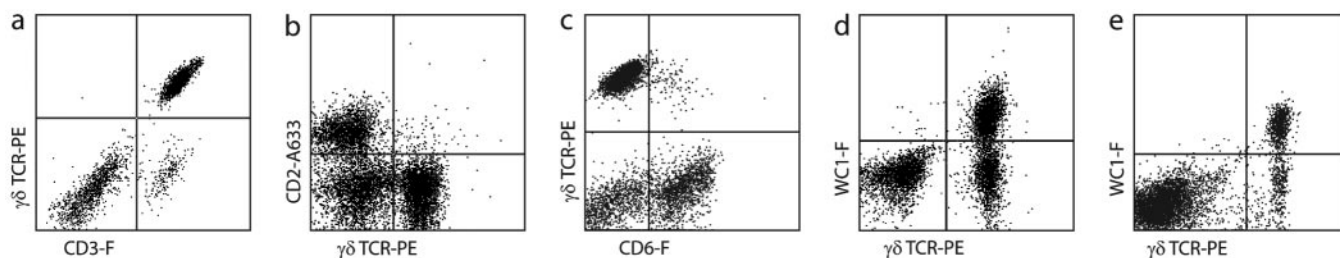

Fig. 1. Phenotype of γδ T cells in pseudoafferent lymph and blood. Flow cytometric analysis of total pseudoafferent lymph cells or PBMC of Animal 001438. In all panels, live cells were gated based on forward/side scatter. γδ T cells form 93% of the CD3+ population and 63% of the total leukocyte population in pseudoafferent lymph (a). These γδ T cells are CD2-negative (b), 0.9% is CD6-positive (c), and 59% is WC1-positive (d). In a paired blood sample, 65% of the γδ T cells are WC1-positive (e).

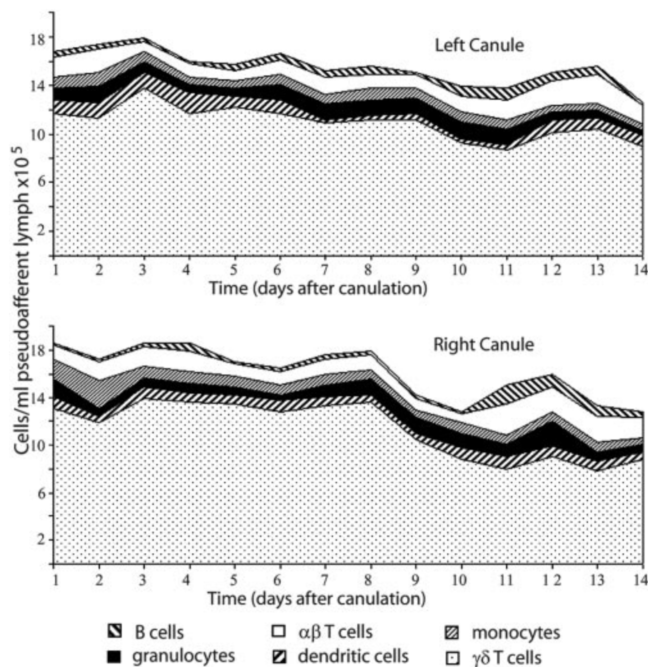

Fig. 2. Lineages of leukocytes present in pseudoafferent lymph. During a period of 14 days, ex vivo pseudoafferent lymph cells were collected three times per day, counted, and stained with lineage markers, as described, prior to flow cytometric analysis. Live cells were gated based on forward/side scatter. The values of three daily time-points were averaged. Per lineage, the average cell numbers per ml pseudoafferent lymph are cumulatively plotted.

lated in human Vδ2+ γδ T cells when they are activated [4], are not expressed by the migrating γδ T cells. These data together suggest that the steady-state phenomenon we describe here is distinct from inflammation-induced effector cell or APC-like behavior, although it may well be that if the bovine skin γδ T cells are activated in some way, they change their migratory behavior and phenotype. During homeostatic conditions, the bovine pseudoafferent γδ T cells probably migrate via the blood back to the skin, with minimal interaction in the lymph node. The reason for this cell traffic is unknown, but the

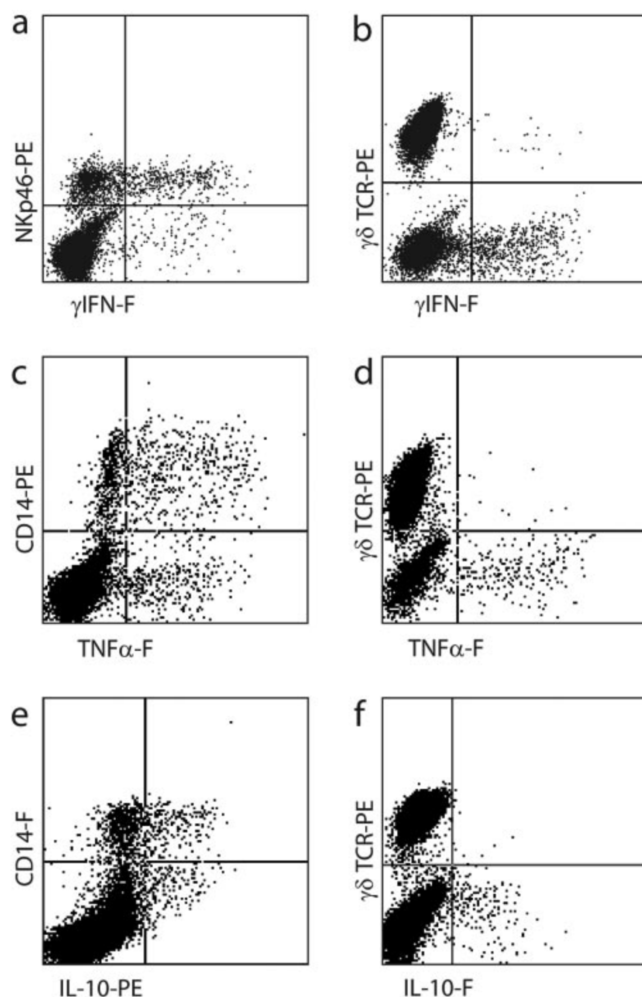

Fig. 4. Cytokine production in pseudoafferent lymph. Pseudoafferent lymph cells, which were stimulated for 6 h with PMA and ionomycin in the presence of Brefeldin A showed substantial IFN-γ production, which was mostly in cells positive for the NK marker NKp46 (a) and almost absent in the γδ T cell population (b). Most of the TNF-α-producing cells were CD14+ cells, and almost none were γδ T cells (c–d), which also holds for IL-10 (e and f).

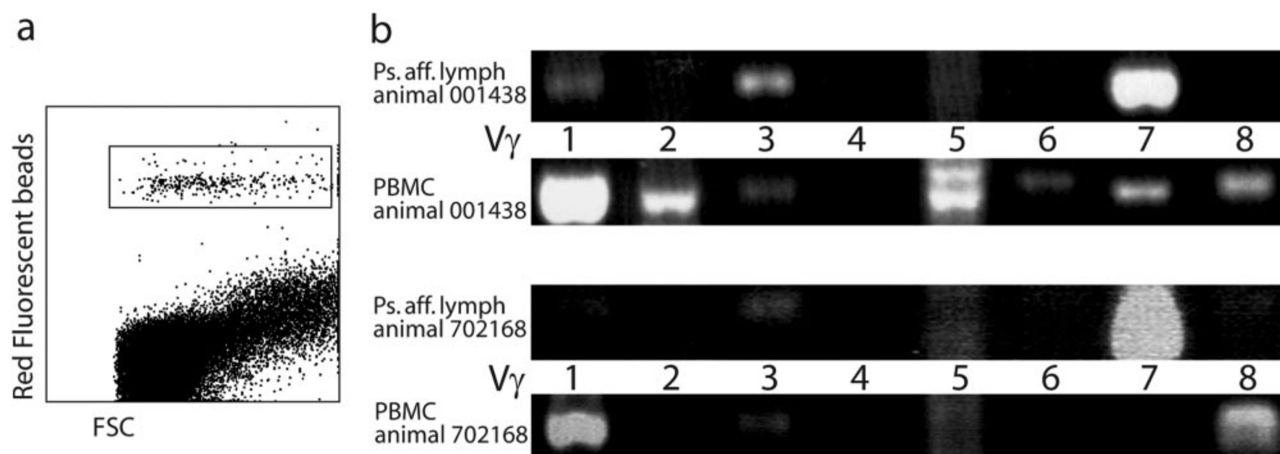

Fig. 3. Collected lymph was draining the skin and is enriched for Vγ segments that are expressed in skin. Flow cytometric analysis of pseudafferent lymph cells (without any immunofluorescent labeling), which were collected 0–8 h after s.c. injection of red fluorescent beads (a). FSC, Forward-scatter. PCR with Vγ-specific forward primers and Cγ universal reverse primer on paired samples of cDNA isolated from pseudoafferent lymph cells and PBMC (b).

fact that the cells migrate in such huge numbers may reflect significant physiological relevance of this phenomenon. One possible function of T cell trafficking to and from organs with large epithelial surfaces is redistribution of T cells. To protect the organism against a harmful agent along the full length of the intestine or at the full surface of the skin, it is necessary to redistribute T cells that may have expanded locally in response to the presence of a harmful agent in their microenvironment. It has been suggested that recirculation via the blood is how redistribution of T cell clones along the gut takes place [28–30].

In addition to different trafficking properties, there are some other important differences between murine and bovine skin γδ T cells. Bovine cells are located in the dermis, and their murine counterparts are located in the epidermis. Murine DETC are almost clonal, but the bovine population is not. Understanding the differences between the skin γδ T cells of these two species may help appreciate the possible different functions of epithelial γδ T cells in general. Homologous functions should not be assumed based on tissue localization only, and this may also hold for other epithelial γδ T cell populations such as intestinal γδ T cells, which are present in many species.

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