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Homing and Function of Human Skin γδ T Cells and NK Cells: Relevance for Tumor Surveillance

Lisa M. Ebert,1 Simone Meuter, and Bernhard Moser2

Normal (noninflamed) human skin contains a network of lymphocytes, but little is known about the homing and function of these cells. The majority of αβ T cells in normal skin express CCR8 and produce proinflammatory cytokines. In this study we examined other subsets of cutaneous lymphocytes, focusing on those with potential function in purging healthy tissue of transformed and stressed cells. Human dermal cell suspensions contained significant populations of Vδ1+ γδ T cells and CD56+CD16− NK cells, but lacked the subsets of Vδ2+ γδ T cells and CD56+CD16+ NK cells, which predominate in peripheral blood. The skin-homing receptors CCR8 and CLA were expressed by a large fraction of both cell types, whereas chemokine receptors associated with lymphocyte migration to inflamed skin were absent. Neither cell type expressed CCR7, although γδ T cells up-regulated this lymph node-homing receptor upon TCR triggering. Stimulation of cutaneous Vδ1+ γδ T cell lines induced secretion of large amounts of TNF-α, IFN-γ, and the CCR8 ligand CCL1. In contrast to cutaneous αβ T cells, both cell types had the capacity to produce intracellular perforin and displayed strong cytotoxic activity against melanoma cells. We therefore propose that γδ T cells and NK cells are regular constituents of normal human skin with potential function in the clearance of tumor and otherwise stressed tissue cells. The Journal of Immunology, 2006, 176: 4331–4336.

Under conditions of homeostasis, human skin contains an extensive network of leukocytes, including dendritic cells (DCs), T cells, NK cells, mast cells, and macrophages (1–5). These leukocytes presumably function together in maintaining skin integrity by continuously surveying for the presence of foreign Ags or signs of cellular stress. The best-characterized mechanism of skin protection involves cutaneous DCs (Langherans cells and dermal DCs), which migrate to skin-associated LNs for the initiation of tolerance or, in the case of skin infection, antimicrobial immune responses (4, 5). In this manner, microbespecific T cells acquire cell surface molecules for guidance to affected skin to combat local infectious agents. Address cues for effector T cell migration to inflamed skin include E-selectin ligand (known as cutaneous lymphocyte-associated Ag/CLA in humans), as well as certain receptors for chemokines expressed in inflamed skin, including CCR4, CCR6, and CCR10 (6–9). Thus, the mechanisms by which effector T cells are recruited from blood to inflamed skin are relatively well understood.

In contrast, we are just beginning to understand the migration properties and function of T cells and other lymphocytes within healthy tissue (10, 11). We recently identified the chemokine receptor CCR8 as a specific marker of αβ T cells present in normal skin and demonstrated that CCL11/309, the unique ligand for CCR8, was constitutively produced at strategic sites in noninflamed skin (12). These cutaneous αβ T cells secreted large amounts of proinflammatory cytokines and may thus contribute to memory responses after contact with previously encountered Ags. However, αβ T cells are unlikely candidates for surveillance against tumors and stressed tissue cells (12). Therefore, we turned our attention to γδ T cells and NK cells. In contrast to αβ T cells, γδ T cells recognize Ags without the need for processing and presentation by classical MHC molecules (13, 14). The major subset of γδ T cells in human peripheral blood expresses the Vδ2 gene segment and recognizes nonpeptide Ags of mostly microbial origin. In contrast, most γδ T cells in epithelial tissues express the Vδ1 gene segment and respond to stress-associated factors, including heat shock proteins and the MHC-related molecules MHC-I-related protein A (MICA) and MHC-I-related protein B (MICB), which are induced on tumor and virus-infected cells (13–16). NK cells similarly recognize abnormal cells using a repertoire of receptors that detect stress-associated molecules as well as the loss of surface MHC protein (17).

Murine skin harbors a major population of Vδ1+ γδ T cells, known as dendritic epidermal T cells (DETCs), which appear to play a critical role in tumor surveillance, as evidenced in γδ T cell-deficient mice (18, 19). Surprisingly, however, a counterpart of DETCs does not exist in human epidermis (1), and it is thus unclear what types of lymphocyte mediate equivalent immune surveillance functions in human skin. In this study we demonstrate that normal human dermis contains distinct populations of γδ T cells and NK cells, both of which express receptors for homing to noninflamed skin and for recognition of allogeneic tumor cells. Together, these cells are likely to make a unique contribution to the elimination of cutaneous tumor and otherwise stressed cells.

Materials and Methods

**Abs, ELISA, and flow cytometry**

Fluorochrome-conjugated Abs to human proteins were purchased from the following sources: rat Ab to CLA (HECA-452), mouse Abs to CD3 (UCHT1), CD16 (3G8), CD45 (HI30), CD56 (B159), pan-TCR-γδ (11F2), Vδ2 (B6.1), perforin (d9), CCR4 (1G1), and CCR6 (11A9) were obtained from BD Pharmingen. Mouse Ab to Vδ1 (T8.2) was purchased from Endogen (Perbio Sciences), and mouse Abs to CD94 (DX22) and NKG2D
T cell clones was determined after stimulation with PMA (20 ng/ml) and formed as previously described (20). The cytokine production capacity of a number of polystyrene beads. Calcein release cytotoxicity assays were performed 2 h before the assay. Migrated cells were collected from the lower compartment of a 5-μm pore size polycarbonate filter (Corning Costar) using cells recovered at 37°C for 3 wk. The two T cell subsets largely shared a selection of tissue-homing receptors associated with T cell homing to inflamed skin (CCR4 and CCR6) (10, 11, 24) were also absent. As an internal control (12), the expression of CLA, and only minor fractions, or none at all, expressed CCR4, CLA, and only minor fractions, or none at all, expressed CCR4, whereas only a minor subset is CD16+. In skin, however, the CD56+ NK cells uniformly lacked CD16 (Fig. 1c), indicating a difference to the majority of circulating NK cells.

A subpopulation of cutaneous NK cells expresses receptors for homing to noninflamed skin

Based on our previous study, CCR8 and its chemokine ligand CCL1 were proposed to provide a selective homing mechanism for the entry of αβ T cells to the skin under steady-state conditions (12). As illustrated in Fig. 2a, a population of skin NK cells also expressed CCR8. These cells differed from the majority of CCR8− NK cells by their low side scatter profile, suggesting that the CCR8+ NK cells have reduced granularity. These two NK cell populations also differed in the expression of CLA, which was coexpressed with CCR8 on NK cells with low side scatter. The significance of the inverse relationship between CCR8 and CLA expression, on the one hand, and side scatter characteristics, on the other hand, is presently unclear. All skin NK cells, regardless of side scatter characteristics, lacked the lymph node (LN)-homing chemokine receptor CCR7. CCR8 on NK cells was functional, because it enabled freshly isolated cells to migrate toward CCL1 in a dose-dependent manner (Fig. 2b). This is in clear contrast to NK cells in peripheral blood, which completely lack CCR8 (Fig. 2c), but a subset of which express CCR7 (22).

Cutaneous γδ T cells display skin-homing properties

Peripheral blood γδ T cells demonstrate inflammatory migration features and, thus, differ from the majority of blood αβ T cells, which express CCR7 for continuous recirculation through secondary lymphoid tissues (23). In human skin, however, we found that the two T cell subsets largely shared a selection of tissue-homing receptors. Flow cytometric analysis (Fig. 3a) revealed that approximately half (53.1 ± 6.9%) of the γδ T cells in normal skin express CCR8, and almost all cells (88.9 ± 7.7%) were positive for CLA. In contrast, CCR7 was not detectable, and two chemokine receptors associated with T cell homing to inflamed skin (CCR4 and CCR6) (10, 11, 24) were also absent. As an internal control (12), the majority of cutaneous αβ T cells were positive for CCR8 and CLA, and only minor fractions, or none at all, expressed CCR4, CCR6, or CCR7 (Fig. 3a). In comparison with skin, γδ T cells in peripheral blood lacked CCR8, and only a few cells were positive for CCR6.

**Statistical analysis**

Data given in the text are presented as the mean ± SEM. Statistical significance was assessed using unpaired Student’s *t* test, and *p* < 0.05 was considered significant.

**Results**

**Vδ1+ γδ T cells and CD16− NK cells in normal human skin**

Normal skin tissue from patients undergoing mammareduction or abdominoplasty surgery was digested to release the dermal cells into suspension, which was subsequently analyzed by flow cytometry. Among the CD45+ leukocytes, populations of γδ T cells and NK cells could be readily identified (Fig. 1a). On the average, from six skin samples, 3.8 ± 1.1% of skin leukocytes were (CD3+γδ TCR−) γδ T cells, whereas 10.6 ± 0.9% were (CD3−CD56+) NK cells. γδ T cells represented 4.7 ± 1.1% of the total cutaneous CD3+ T cells (Fig. 1b), similar to previous estimates based on immunohistochemical studies (1). The Vδ1− subset of γδ T cells predominated in normal human skin, because an Ab to the Vδ1 chain, but not an Ab to the Vδ2 chain, stained virtually identical numbers of cells, as detected with an Ab to pan-TCR-γδ.

Based on the expression of CD16, NK cells are divided into two subpopulations (21). The majority of peripheral blood NK cells express CD16, whereas only a minor subset is CD16−. In skin, however, the CD56+ NK cells uniformly lacked CD16 (Fig. 1c), indicating a difference to the majority of circulating NK cells.

**Functional assays**

Four-hour Transwell chemotaxis assays were performed using a 5-μm pore size polycarbonate filter (Corning Costar) using cells recovered at 37°C for 2 h before the assay. Migrated cells were collected from the lower compartment and stained and quantified by flow cytometry relative to a fixed number of polystyrene beads. Calcein release cytotoxicity assays were performed as previously described (20). The cytokine production capacity of T cell clones was determined after stimulation with PMA (20 ng/ml) and ionomycin (1 μM) for 24 h at a cell concentration of 1.6 × 10^6/ml.
a

![Graph showing CCR8, CLA, and CCR7 levels](image)

**FIGURE 2.** A subset of cutaneous NK cells expresses CCR8 and CLA and migrates toward CCL1. *a,* Dermal cell suspensions were analyzed by flow cytometry, gating on CD45^+ CD3^+ CD56^+ cells, and the intensity of staining for CCR8, CLA, or CCR7 was plotted against the side-scatter profile (n = 3–4). *b,* Skin cells were subject to Transwell migration assays using CCL1 (○) or medium alone (○), and total migrated NK cells (CD3^- CD56^+) were enumerated using flow cytometry relative to a standard number of polystyrene beads. *c,* PBMC were analyzed by flow cytometry, gating on CD3^- CD56^+ NK cells (n = 4).

for CLA (Fig. 3b), underscoring the unique nature of skin-resident γδ T cells.

**Migration and functional properties of cultured, skin-derived γδ T cells**

The yield of primary γδ T cells isolated from noninflamed skin tissue did not allow a detailed investigation; therefore, functional and phenotypic studies were performed with a panel of skin-derived γδ T cell clones. Similar to freshly isolated skin γδ T cells, approximately half the clones expressed readily detectable levels of CCR8 (>20% CCR8 positivity; Fig. 4a, left panel). Under the present culture conditions, CCR8^- clones did not up-regulate the expression of this chemokine receptor, whereas CCR8^+ clones maintained their respective levels, suggesting that CCR8 is a stable feature in distinct cutaneous γδ T cells. As with αβ T cells (12), the conditions required for induction of CCR8 expression in CCR8^- γδ T cells are not known. Chemotaxis assays confirmed that CCR8 on receptor-positive γδ T cell clones was functional, as shown previously for αβ T cell clones (12) (data not shown). In contrast, only one of the 21 clones examined expressed CCR4 (>20% CCR4 positivity; Fig. 4a, right panel), which is in keeping with the lack of skin-associated inflammatory chemokine receptors on fresh isolated cutaneous γδ T cells. Interestingly, PHA activation of γδ T cell clones resulted in a transient loss of cell surface CCR8 (Fig. 4b). This suggests that γδ T cells temporarily alter their homing properties after local activation and may no longer be retained in the skin environment, but instead gain responsiveness to alternative chemokines (25). In keeping with this concept, CCR7 was strikingly up-regulated on day 3 after activation (Fig. 4b), followed by complete down-regulation as cells returned to a resting state (not shown). This observation suggests that locally activated cutaneous γδ T cells can assist adaptive immune responses after relocation to the draining LNs, as proposed recently for blood γδ T cells (26).

All clones tested produced very high levels of the proinflammatory cytokines TNF-α (7–43 ng/ml) and IFN-γ (94–1485 ng/ml) after stimulation with PMA and ionomycin (Fig. 4c). Additionally, they secreted large amounts of CCL1 (6–81 ng/ml), suggesting that CCL1 was responsible in part for the transient loss of cell surface CCR8.

**Skin γδ T cells and NK cells have the capacity to lyse melanoma cells**

To address the potential role of cutaneous γδ T cells and NK cells in tumor surveillance, their ability to mount cytolytic responses against melanoma cells was examined. First, skin-derived γδ T cell clones were tested in cytotoxicity assays using heterologous melanoma cell lines as targets. Of seven clones tested, five exerted robust cytotoxic responses against the melanoma cell line SK-Mel2, resulting in up to 90% target cell death at an E:T cell ratio of 25:1, whereas two clones failed to do so (Fig. 5a, left panel). Similar results were observed with the melanoma cell line HS-294 (not shown). In control experiments using skin-derived αβ T cell clones (six CD8^- clones and four CD4^- clones), eight clones completely lacked lytic activity against SK-Mel2 and HS-294 cells (not shown), and only two clones showed borderline activity (Fig. 5a, right panel). These data are not surprising, because the mode of Ag recognition differs strikingly between αβ T cells and γδ T cells (13, 14), and the frequency of memory CD8^- αβ T cells with alloseactivity is relatively low. Major mechanisms contributing to lymphocyte-mediated cytotoxicity involve perforin, which is released at the contact zone between effector and target cells (27), and activating NK receptors, such as NKG2D, which recognizes MHC-I-related MICA and MICB as well as other stress-related molecules (15, 17). Perforin was not detected in freshly isolated skin T cells (Fig. 5b). However, all 12 γδ T cell clones tested contained detectable levels of perforin, whereas only two of 26 skin-derived αβ T cell clones were positive (Fig. 5c). The lack of perforin in skin αβ T cell clones is in line with their proposed involvement in the control of memory responses rather than tissue cell lysis (12). In contrast to αβ T cells, freshly isolated γδ T cells were uniformly positive for NKG2D and maintained this activating receptor during subsequent cell culture (Fig. 5d). NKG2D signaling in NK cells and γδ T cells leads to direct cell activation that may result in target cell lysis, whereas cytotoxicity in CD8^- αβ T cells is largely controlled by their clonal TCRs recognizing specific tumor Ags (28, 29). Collectively, these data suggest that γδ T cells present in normal skin have the potential to eliminate stressed cells, including tumor cells.

The cytotoxic capacity of cutaneous NK cells was examined in cultured cells after expansion of CD45^- skin leukocytes for 2–3 wk in the presence of IL-2, followed by purification of CD3^- CD56^- NK cells by cell sorting. These lines demonstrated clear cytotoxic activity against SK-Mel2 cells, with an average of 75% target cell lysis at an E:T cell ratio of 25:1 (Fig. 6a). Furthermore, these cell lines were uniformly positive for intracellular perforin (Fig. 6b, right panel). Again, as noted for skin γδ T cells, freshly isolated skin NK cells completely lacked this lytic marker (Fig. 6b, left panel). Remarkably, and in clear contrast to γδ T cells, NKG2D could not be detected on freshly isolated skin NK cells, and its induction of expression required previous NK cell culture in the presence of IL-2. Obviously, NK cells in healthy human skin are devoid of immediate lytic activities, which is in
keeping with a previous study demonstrating that the CD16− subset of NK cells within LNs fully depends on activation for mounting lytic activity (30).

**Discussion**

Local immune surveillance for tumors and stressed tissue cells is crucial for the maintenance of skin homeostasis. In mice, this function is largely fulfilled by DETCs in the epidermis (18, 19). However, because human epidermis lacks DETCs, it has remained unclear how these important functions are performed in humans. In this study we demonstrate that human dermis contains significant populations of γδ T cells and NK cells that express receptors for homing to normal skin and, in relation to their state of activation, receptors for tumor cell killing. Although the presence of Vδ1+ γδ T cells in the human dermis has been previously documented (1), their function and migration properties have remained elusive. Their unique TCR repertoire indicates that they are not a random sample from circulating γδ T cells (31), a view supported by our finding that cutaneous, but not blood, γδ T cells expressed the skin-homing receptors CCR8 and CLA. Human dermal γδ T cells uniformly expressed the activating receptor NKG2D for killing of tumor or otherwise stressed cells (17). Although freshly isolated cutaneous γδ T cells lacked perforin, they acquired it upon cell activation, in keeping with the observation that murine DETCs require activation to develop cytotoxic function against melanoma (32). In many respects, dermal γδ T cells resemble Vδ1+ γδ T cells in human intestinal epithelium, which also killed heterologous intestinal tumor cells by a process involving MHC-related MICA and MICB and probably other stress-related molecules (15, 33, 34).

Similar to γδ T cells, the nature and function of NK cells present in normal human skin are ill defined. We show in this study that these cells constitute a significant fraction of dermal leukocytes. Of note and in clear contrast to NK cells in peripheral blood and LNs (17, 30), NKG2D is completely absent in cutaneous NK cells, but is strongly induced upon in vitro culture. Similarly, perforin is present in cultured, but not freshly isolated NK cells. Obviously, healthy human dermis is devoid of NK cells with the capacity for immediate target cell lysis. The majority of cutaneous NK cells with a low side-scatter profile expressed both CCR8 and CLA, but lacked CCR7 and therefore displayed a homing phenotype highly reminiscent of cutaneous αβ and γδ T cells, but clearly different from that of NK cells in peripheral blood (21, 22, 30). The major subset of CD16+ NK cells in blood expresses CXCR1 and CX3CR1 for homing to inflammatory sites and contains perforin for immediate cytotoxic effector function. In contrast, the minor subset of CD16+ NK cells expresses CD62L and CCR7 for recirculation through LNs and has no intracellular perforin. Cutaneous NK cells also lack CD16 and perforin expression, but differ from blood CD16+ NK cells with respect to migration properties. Interestingly, normal small-intestinal epithelium was also recently shown to harbor a population of NK cells (35). Thus, in analogy to memory αβ T cells (11, 24), subsets of CD16+ NK cells with individual homing preferences for normal peripheral tissues may exist.

In contrast to the rapid recruitment of effector lymphocytes to sites of skin inflammation (9, 24), the mechanisms controlling the steady-state traffic of memory T cells and their maintenance within healthy tissue are not well understood (10, 11). CCL1, the single ligand for CCR8, is produced in normal healthy skin, notably by...
vascular and perivascular cells within superficial dermal plexus and by epidermal Langerhans cells and melanocytes (12). This chemokine system may regulate the recruitment and cutaneous positioning of blood CCR8 \(^\textsuperscript{-}\) αβ T cells and, as proposed in this study, a large fraction of cutaneous γδ T cells and NK cells. Yet, we still have little knowledge about the traffic parameters and the life span of cutaneous lymphocytes (36, 37). The scarcity of CCR8 \(^\textsuperscript{+}\) lymphocytes within peripheral blood may indicate that the skin is their primary site of residence. In preliminary coculture experiments, we have noticed that dermal fibroblasts supported the slow proliferation of freshly isolated cutaneous T cells and NK cells (L. M. Ebert and B. Moser, unpublished observations). Skin is a rich source of growth factors and, in analogy to bone marrow (38), may support homeostatic expansion in local lymphocytes. It is possible that skin lymphocyte numbers are maintained by several factors, including rates of skin recruitment and exit as well as the rates of local survival and proliferation.

Distinct lymphocyte populations within healthy skin are likely to play a crucial role in local immune surveillance. Skin-tropic memory αβ T cells, also referred to as peripheral immune surveillant T cells (11, 12), may contribute to memory responses against previously encountered pathogens. However, cytotoxic peripheral immune surveillance T cells with selectivity for skin tumors appear to be rare, suggesting that they may not participate in tumor surveillance. Our present study has characterized for the first time populations of human skin-homing γδ T cells and NK cells with the potential to elaborate lytic functions, which may involve molecules commonly associated with situations of tissue stress, such as those present on transformed or virally infected cells (13–17). Furthermore, we have shown that these three distinct subsets of cutaneous lymphocytes share a common address code, enabling their colocalization within noninflamed skin. Due to their specialization in Ag recognition and function, these diverse cell types may cooperate in cutaneous immune surveillance against a wide variety of challenges.

**FIGURE 5.** γδ T cells in normal skin express NKG2D and lyse melanoma cells after activation. *a*, Skin-derived γδ (left panel) or αβ (right panel) T cell clones were tested in calcein release cytotoxicity assays using the melanoma cell line SK-Mel2 as targets. Values represent the mean and SEM calculated from triplicate wells for each clone. *b*, The expression of perforin in freshly isolated skin T cells was assessed by flow cytometry after gating on CD3 \(^+\) cells. *c*, The expression of intracellular perforin was determined for γδ (left panel; n = 12) and αβ (right panel; n = 26) T cell clones by flow cytometry (open histograms). *d*, Flow cytometric analysis of cell surface NKG2D in γδ T cells and αβ T cells (open histograms), either immediately after isolation from normal human skin tissue or after polyclonal expansion during cell culture (n = 2). Filled histograms in *c* and *d* represent staining with isotype-matched control Abs.

**FIGURE 6.** NK cells in normal skin lack NKG2D and perforin, but lyse melanoma cells after activation. *a*, NK cells were sorted from IL-2-expanded skin leukocyte cultures and tested in cytotoxicity assays using SK-Mel2 cells. Values are the mean and SEM calculated from triplicate wells for cell lines derived from three different donors. *b*, Expression of intracellular perforin was determined for NK cells, either freshly isolated (open histograms; left panel) or after expansion in IL-2 (right panel) by flow cytometry (n = 3). *c*, Flow cytometric analysis of cell surface NKG2D (open histograms) in freshly isolated (left panel) and cultured (right panel) NK cells (n = 2). Filled histograms in *b* and *c* represent staining with isotype-matched control Abs.

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**Disclosures**

The authors have no financial conflict of interest.

**References**


