

INVESTIGATIVE REPORT

Natural Killer Cells in Human Peripheral Blood and Primary Cutaneous Natural Killer Cell Lymphomas May Express Cutaneous Lymphocyte Antigen

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In normal human peripheral blood, cutaneous lymphocyte antigen is expressed by memory T cells, suggesting a specific tissue-homing population of T cells. In this study it is demonstrated that 6% of CD56+ natural killer cells in peripheral blood also express cutaneous lymphocyte antigen (CLA). It was also detected that most of the tumor cells in primary cutaneous nasal-type natural killer cell lymphomas were CLA-positive, whereas primary nasal natural killer cell lymphomas were CLA-negative. Although natural killer cells traditionally are known to be non-specific immune cells without antigen specificity and little is known about the role of natural killer cells in skin diseases, the results of this study suggest the existence of a subset of skin-associated CLA+ CD56+ natural killer cells. These natural killer cells may be related to the pathogenesis of primary cutaneous natural killer cell lymphomas. Key words: cutaneous lymphocyte antigen; natural killer cells; primary cutaneous natural killer cell lymphomas.

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Memory T cells that infiltrate the skin express a unique skin-homing receptor called the cutaneous lymphocyte antigen (CLA), a carbohydrate (HECA-452) epitope on the P-selectin glycoprotein ligand-1 that facilitates the targeting of T cells, especially CD4+ T-helper cells to inflamed skin (1). CLA is highly expressed in the majority of skin-infiltrating T cells in benign inflammatory skin diseases and cutaneous T-cell lymphoma (CTCL) (1–4). In addition, circulating neutrophils and monocytes and cultured blood dendritic cells are shown to express CLA (4). Our previous work concerned primary cutaneous natural killer cell lymphoma (NKL), which is more prevalent in Asia, and incidentally we detected high expression of CLA in the tumor cells of primary cutaneous NKL (5).

Tissue-specific homing in natural killer (NK) cells is not known and NK cells are defined functionally by their ability to lyse target cells without prior sensitization and major histocompatibility restriction (6). A few studies have been conducted regarding the role of NK cells in skin diseases, such as alteration of NK cell number and activity in atopic dermatitis and viral infections (7–10). NK cells have been found in the cellular infiltrates in lesional psoriatic skin (11).

Our objective was to elucidate the CLA+ skin-homing NK cell population in normal peripheral blood and any possible association with skin diseases, particularly NKLs occurring primarily in the skin.

MATERIAL AND METHODS

Clinical data

Eight cases of primary cutaneous (initially presenting at skin without other organ involvement within 6 months) NKL (5, 12) without clonal rearrangement of the T-cell receptor (TCR) gamma gene were identified at the Department of Dermatology. The diagnosis was consistent with the previous description of extranodal nasal/nasal type NK/T-cell lymphoma by WHO classification. Our cases occurred primarily in the skin and we therefore use the term “primary cutaneous NKL” in this study. The study group included 3 males and 5 females, mean age 42 years (range, 16–77 years). All the patients had recurrent benign-looking subcutaneous inflammatory nodular lesions with a predominantly subcutaneous lymphoid infiltrate. The lower extremities were the sites of predilection. Seven patients died within 43 months of onset of the skin lesions.

One case of primary nasal NKL (also categorized as extranodal nasal/nasal type NK/T-cell lymphoma by the WHO classification) *without* skin involvement was selected for comparison of CLA expression. Myelomonocytic leukemia was excluded in all patients, since the infiltrates did not express any myelocytic markers and were negative to CD34.

Immunohistochemical study and genotypic analysis of TCR

Immunohistochemical analyses were performed using paraffin-embedded skin specimens. The skin specimens were obtained from the 8 cases of primary cutaneous NKL. Antibodies for CD3 (Dako, Glostrup, Denmark), CD20 (Dako), CD45RO (Dako), Ki-67 (Immunotech, Marseilles, France), TIA-1 (Dako), CD4 (Dako), CD8 (Dako), Tdt (Dako), CD56 (Becton-Dickinson, Mountain view, CA, USA) and CLA (Becton-Dickinson) were used following the standard streptavidin-biotin peroxidase

method. The percentage of staining was graded by the percentage of tumor cells and the scoring scale was as follows: -, none; +, less than 10%; 2+, 10–25%; 3+, 25–50%; 4+, 50–75%; 5+, 75–100%. Genotyping analysis of the TCR- γ gene by polymerase chain reaction (PCR) was performed using primer sets directed against all TCR- γ variable regions and joining regions in all skin biopsy samples. PCR of the beta-globin gene was also performed to assure the quality of DNA.

FACS analysis

Peripheral blood samples from healthy volunteers aged 28–31 years (4 males and 4 females) were taken. Peripheral blood mononuclear cells (PBMC) were prepared by centrifugation over Ficoll-Paque plus. The PBMC samples were double-stained with fluorescein isothiocyanate (FITC)-labelled CLA (HECA-452, PharMingen, San Diego, CA, USA) antibody and Phycoerythrin (PE)-labelled CD3, CD4, CD8 and CD56 antibodies (Becton-Dickinson). Isotype-matched irrelevant antibodies were used as negative controls. Using a FACS machine (Becton Dickinson, San Jose, CA, USA), after gating on the lymphoid cell population by side and forward light scatter, different cell subsets (CD3, CD4, CD8 and CD56) were calculated for expression of CLA in dual staining. Since there is also a minor population of T cells (CD3+CD8+) that express CD56, each CD3+CD56+CLA+ population and CD8+CD56+CLA+ population was also calculated by triple analysis. For triple analysis, FITC-labelled CLA, Percp-labelled CD3 (Dako, CA, USA), Percp-labelled CD8 (Dako, CA, USA) and PE-labelled CD56 were used.

RESULTS

Immunohistochemical results of 8 cases of primary cutaneous natural killer cell lymphoma

The 8 cases analyzed presented with skin lesions such as subcutaneous nodules or plaques and with no evidence of systemic lymphomas within 6 months of diagnosis. Genotyping analysis of the TCR- γ gene using PCR did not demonstrate any monoclonality in the skin of any of the 8 cases (Table I).

CLA expression was demonstrated 4+–5+ in the CD56+ tumor cells forming subcutaneous lymphoid infiltrate in the 8 cases (Figs 1A and B). The proportion of CLA+ cells was higher than in dermal angiocentric

infiltrate. In contrast, few positive cells were detected in the case of primary nasal NKL (Fig. 1C).

The immunophenotype was similar; positive for cytoplasmic CD3 (6 cases; 4+–5+), CD45RO (6 cases; 5+), CD56 (8 cases; 5+), TIA-1 (8 cases; 4+–5+), Ki-67 (8 cases; 3+–5+). The lymphoma cells were negative for CD20, CD8 and TdT. CD30 was positive (2+–3+) in 2 cases and CD4+ (2+–3+) in 2 cases.

FACS results of PBMC from 8 healthy volunteers

CLA was expressed by a small but consistent proportion of CD4+ T cells, CD8+ T cells and CD56+ cells. CLA expression was as follows (mean \pm SD): 13.3 \pm 3.2% in CD3+ cells; 21.9 \pm 3.3% in CD4+ cells; 6.0 \pm 1.6% in CD8+ cells; and 6.3 \pm 1.3% in CD56+ cells. Representative figures can be seen in Fig. 2.

In subject number 1, the population of CD3+CD56+CLA+ cells was only 1.9% and CD8+CD56+CLA+ was 0.6% (Table II).

DISCUSSION

Continuous recirculation of cells from blood through tissue (lymphoid and other tissues) and back through the lymphatics to the blood is a key characteristic in the immune system (1–3, 13). For efficient immune surveillance, the cells of the immune system are specifically targeted to sites of inflammation or sites of priming (1–3, 13). Following antigen encounter and recognition in an appropriate microenvironment, lymphocytes differentiate into effector/memory cells acquiring the ability to access extralymphoid immune effector sites where they are most likely to re-encounter their specific antigen. Distinct subsets of memory/effector cells exist with tissue-selective patterns of migration and adhesion molecules, termed “homing receptors”, that mediate lymphocyte binding to high endothelial venules in different lymphoid organs (13–16).

CLA is known as a skin-homing receptor expressed on a minority of memory-type peripheral blood T cells (13–16). CLA is highly expressed in the skin-infiltrating

Table I. Results of immunohistochemical analysis of primary cutaneous natural killer cell lymphoma.

Case no.	CD3 (cytoplasmic)	CD4	CD8	CD45RO	CD20	CD30	CD56	TIA-1	Ki-67	CLA
1	4+	–	–	4+	–	–	5+	3+	2+	5+
2	4+	2+	–	5+	–	–	4+	4+	2+	4+
3	3+	–	+	4+	–	+	5+	3+	2+	4+
4	+	–	+	4+	–	–	4+	4+	2+	4+
5	–	–	–	4+	–	2+	5+	4+	3+	5+
6	+	3+	–	4+	–	–	5+	3+	3+	5+
7	–	2+	–	4+	–	–	4+	3+	2+	4+
8	+	–	–	4+	–	–	4+	3+	3+	4+

Percentage of staining was graded by a scoring scale: –, none; +, less than 10%; 2+, 10–25% reactivity; 3+, 25–50% reactivity; 4+, 50–75% reactivity; 5+, 75–100% reactivity.

CLA: Cutaneous lymphocyte antigen.

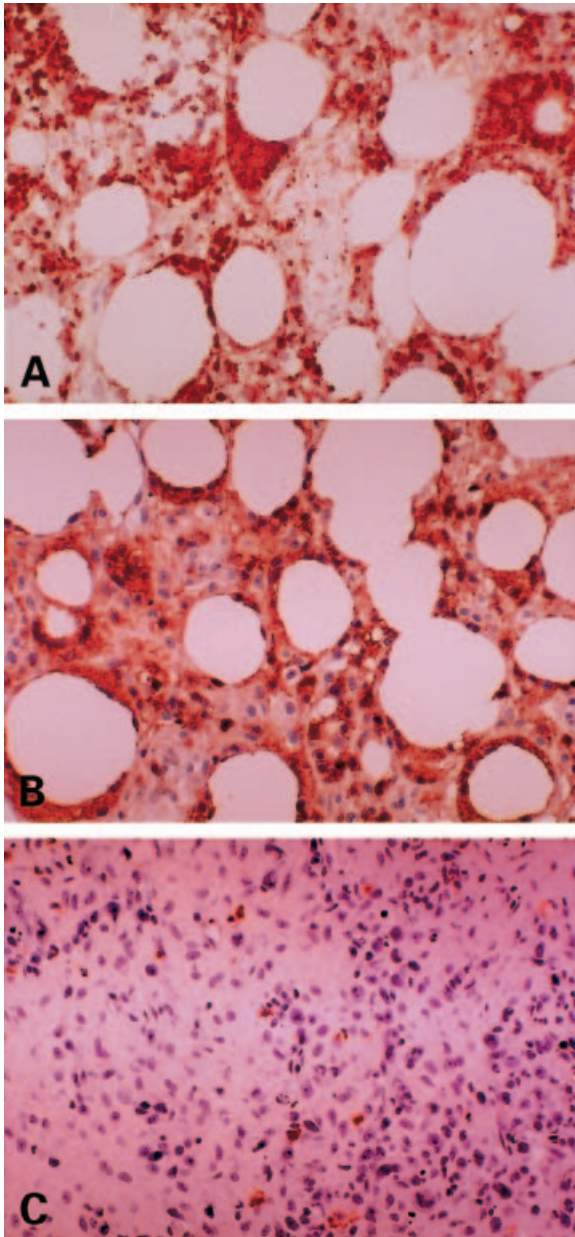


Fig. 1. A biopsy taken from a subcutaneous nodule on the shin in a patient with primary cutaneous natural killer cell lymphoma (NKL) showed lobular panniculitis composed of CD56+ tumor cells (A) (immunoperoxidase staining of CD56, $\times 200$). The CD56+ tumor cells were weakly positive to cutaneous lymphocyte antigen (CLA) (B) (immunoperoxidase staining of CLA, $\times 200$). In contrast, few positive cells were detected in a case of primary nasal NKL (C) (immunoperoxidase staining of CLA, $\times 200$).

T cells in inflammatory skin diseases and CTCL (1–4). In addition, subpopulations of myelomonocytic cells and dendritic cells are shown to express CLA (4, 16), whereas in the peripheral blood, CLA was expressed on $<20\%$ of CD4+, CD8+ and CD56+ NK cells, $>60\%$ of CD4+, CD8+ and CD56+ cells isolated from skin-derived lymph expressed CLA, indicating an important role in cell homing to the skin (13). Our observation that CD56+ NK cells in peripheral

blood of healthy volunteers and the majority of cells in primary cutaneous NKL also express CLA suggests the existence of a subset of CLA+ CD56+ NK cells preferentially homing to the skin. The finding that the case of primary nasal NKL had few CLA+ cells may be further evidence to support this suggestion.

The biological and clinical relevance of human NK cells is not fully known. NK cells comprise 10–15% of human peripheral blood lymphocytes and are defined functionally by their ability to lyse target cells without deliberate prior sensitization and without restriction by major histocompatibility (6). No single surface antigen described unambiguously identifies all human NK cells. The antigens used most extensively as NK cell markers are CD56 and CD16. The CD56 antigen is expressed by virtually all human peripheral blood cells capable of non-MHC-restricted cytotoxicity. Most CD56 peripheral blood cells also express CD16. There are limitations to the use of CD56 alone, although CD56 appears to identify all lymphocytes possessing NK activity (6, 17). NK cells have been implicated in various activities including the destruction of tumor cells, resistance to viral infections and regulation of hematopoiesis (6) but little is known about the role of NK cells in skin diseases. Furthermore, recirculation patterns and specific homing of NK cells have not been studied. Little is known about the expression of CLA on NK cells in skin diseases. A few studies have been conducted concerning the role of NK cells in atopic dermatitis and psoriasis, which are among the most common inflammatory skin diseases. In atopic dermatitis, there was a negative correlation between the percentage of NK cells and total IgE levels (7) and NK cell activity was less than that in normal persons (7–9). Patients with atopic dermatitis do not cope well with certain cutaneous viral infections and this may be related to alteration of NK cells (10). NK cells were found in the cellular infiltrates in lesional psoriatic skin (11).

During the past decade, an important new subtype of non-Hodgkin's lymphoma has emerged and this is the lymphoma derived from NK cells (18–23). The NKL, which can involve the skin in a primary or secondary fashion, is characterized by the expression of the NK-cell antigen CD56. These CD56+ lymphomas are further subdivided into nasal NKL and non-nasal NKL that often arise in extranodal locations, including the skin (18–23). NK cells do not show expression of TCR protein and the TCR is a germ-line configuration (18). NK cells are negative for surface CD3 but can show cytoplasmic positivity of CD3 and other T-cell markers (18–23). These characteristics of NK cells are consistent with NKL. We have observed typical cases of primary cutaneous NKL. As well as CTCL, primary cutaneous NKL can be phenotypically differentiated from populations of non-cutaneous lymphomas. The high expression of the CLA skin homing receptor in

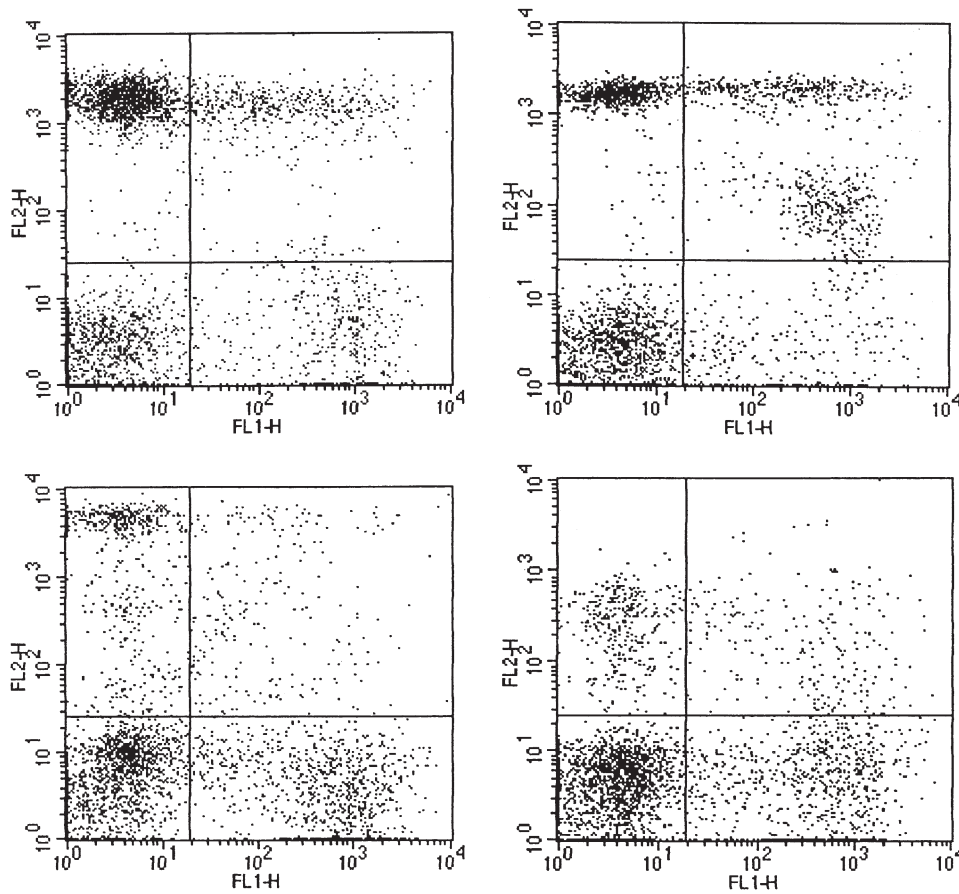


Fig. 2. Representative figure of FACS analysis of cutaneous lymphocyte antigen expression of peripheral blood mononuclear cells of healthy controls. (left upper: CD3+CLA+, right upper: CD4+CLA+, left lower: CD8+CLA+, right lower: CD56+CLA+).

primary cutaneous NKL may support the view that NKL has a unique predilection for skin. CLA positivity in this type of lymphoma is a new finding suggesting that skin disease-related NK cells are associated with the development of primary cutaneous NKL.

As in memory T cells, epidermal dendritic cells (Langerhans' cells) express CLA with preferential migration of skin and skin-associated lymphoid tissue; they migrate from the epidermis through the superficial lymph system to the associated lymph nodes, where they may stimulate naïve lymphocytes. Double immunohistochemical staining showed that 14% of CD68+ dendritic

cells (monocyte/macrophages) and 2% of CD1+ dendritic cells (Langerhans' cells) in normal skin display CLA (24). In one study, further evidence was found of the presence of CLA+ antigen presenting cells (perivascular dermal dendritic cells and epidermis) in the skin depending on stimuli (25). In this manner, a certain proportion of NK cells or other NK-like cells may have a tendency towards skin homing. Our additional study showed that CD3+CD56+CLA+ cells and CD8+CD56+CLA+ cells were only minor populations. Only one article described the CD56+CLA+ cells (about 12.2%) without commenting on any significance (13). Local microenvironment in skin may play an important role in the distribution of immune resources by modulating the expression and function of the CLA of NK cells as well as T cells (24–31); repeated activation in the skin may serve to reinforce CLA expression on NK cells functionally associated with the skin, thus enhancing the functional efficiency of these cells by preferentially focusing their recirculation to the skin or related sites.

In conclusion, our results suggest the existence of skin-associated CLA+ CD56+ NK cells which may be associated with the pathogenesis of variable skin diseases, especially primary cutaneous NKL. However, the clinical relevance of these cells in association with various skin diseases has to be studied further.

Table II. FACS results of cutaneous lymphocyte antigen-positive peripheral blood mononuclear cells of healthy controls.

Subject no.	CD3+ (%)	CD4+ (%)	CD8+ (%)	CD56+ (%)
1	12.9	23.7	7.0	5.1
2	11.5	17.1	5.7	5.4
3	8.2	21.5	2.3	5.7
4	15.9	23.9	7.7	7.5
5	13.1	23.9	6.9	7.7
6	10.9	16.5	5.6	4.5
7	17.2	23.4	6.5	7.4
8	17.0	25.3	6.3	7.1

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