CD7-Negative Helper T Cells Accumulate in Inflammatory Skin Lesions

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Recently, we identified a particular T-cell subset in the peripheral blood of normal individuals that lack CD7 expression. In this study we determined the portion of $CD7^-$ T cells in the peripheral blood and skin of patients with various inflammatory skin diseases. We found that skin-infiltrating lymphocytes isolated from different benign and malignant skin lesions (n = 20) contain a high portion of $CD7^-$ helper T cells, whereas the number of $CD7^-$ T cells in the peripheral blood was not altered compared to healthy controls. Cell activation *in vitro* did not induce CD7 expression in negative T cells but increased CD7 expression in CD7-positive cells. Thus, lack of CD7 expression seems to be a stable characteristic in a major subset of skin-infiltrating lymphocytes. During long-term culture of $CD7^-$ helper T-cell clones derived from a psoriasis skin lesion, no phenotypic change in the CD7 phenotype could be monitored by sequential flow-cytometric analyses. No CD7 mRNA could be detected by Northern blot analysis, indicating transcriptional regulation of CD7 expression. The results show that $CD7^-$ T cells accumulate in certain inflammatory skin lesions without alteration of the circulating $CD7^-$ population. These cells may be identical to or derived from $CD7^-$ T cells of the peripheral blood. *Key words: $CD7^-$ T cells/skin inflammation/cutaneous lymphoma. J Invest Dermatol 102:328–332, 1994*

CD7 is a 40-kilodalton (kD) glycoprotein expressed on the surface of peripheral blood T cells and thymocytes early during T-cell ontogeny [1]. Therefore, CD7 expression is a useful marker for the identification of T-cell malignancies. Although the functional role of the CD7 molecule remains to be elucidated, recent studies indicate that CD7 may be involved in signal transduction and/or may function as an adhesion molecule during T-cell activation [2,3]. $CD7^-$ T cells have been found in a number of pathologic conditions, suggesting that absence of this antigen may have important pathophysiologic consequences. Absence of CD7 expression on T cells was reported to be associated with severe combined immunodeficiency [4]. Furthermore, in contrast to the majority of T-cell malignancies, cutaneous T-cell lymphoma (CTCL) predominantly lacks CD7 expression. This phenomenon is reported to be helpful in differentiating a malignant lymphoma from reactive cutaneous infiltrates [5,6]. Furthermore, leukemic T cells in CTCL, characterized as structurally distinctive T lymphoid cells with a highly convoluted nucleus (Sézary cells), do not express CD7 [7]. With the exception of this antigen, however, Sézary cells express the same marker spectrum as normal mature helper T cells.

Recently, we identified a subpopulation of $CD7^-$ T cells in the peripheral blood of normal donors [8]. Northern blot analysis revealed regulation of CD7 expression at the mRNA level. The majority of circulating $CD7^-$ T cells are of the $CD4^+$ "memory" phenotype. Thus, absence of CD7 on T cells *in vivo* seems to be a stable phenotype in a subset of normal peripheral blood T cells. In this study we determined the portion of $CD7^-$ T cells in the peripheral blood and in the skin of patients with various inflammatory skin diseases. Interestingly, skin-infiltrating lymphocytes (SILs) derived from a variety of benign and malignant skin diseases predominantly consist of T cells that lack CD7 antigen expression. In contrast, the population of $CD7^-$ T cells in peripheral blood is not altered. The results implicate a specific physiologic function of $CD7^-$ helper T cells during inflammatory reactions of the skin.

MATERIALS AND METHODS

Cell Isolation and Culture SILs were isolated from various inflammatory skin lesions (psoriasis vulgaris [n = 7], atopic dermatitis [n = 5], cutaneous T-cell lymphoma [n = 4], lichen planus [n = 2], pityriasis rubra pilaris [n = 1], pityriasis lichenoides et varioliformis acuta [n = 1]) as previously described [9].

Briefly, a punch biopsy was obtained from each patient, subcutaneous adipose tissue was removed, and residual biopsy was devided into several pieces and cultered in RPMI 1640 medium (GIBCO, Berlin, FRG) containing 10% heat-inactivated human pooled male serum (Sigma, Munich, FRG), 2 mM L-glutamine, penicillin 100 IU/ml, streptomycin 100 μg/ml, recombinant human interleukin-2 (IL-2; 100 U/ml, Euro Cetus Corporation, Amsterdam, The Netherlands), 20 Gy irradiated pooled peripheral blood mononuclear cells (PBMCs) as filler cells, and either 5 μg/ml phytohemagglutinin (PHA; Biochrom, Berlin, FRG) or 2.5 ng/ml anti-CD3 antibody (OKT3; Ortho Pharmaceutical, Neckargemünd, FRG) at 37°C, 5% CO_2. During 7–12 d of culture, mononuclear cells migrated out of tissue. Cells were harvested and phenotyped by immunofluorescence analysis.

PBMCs were isolated from heparinized blood (50 IU/ml) of 39 healthy donors and 31 patients with inflammatory dermatoses (psoriasis vulgaris [n = 7] and atopic dermatitis [n = 24]) using standard Ficoll-Isopaque gradient centrifugation.

T-cell–enriched populations were purified from PBMCs by passage through a nylon-wool column (Wako Chemicals, Neuss, FRG). The contamination with $CD14^+$ monocytes and $CD19^+$ B cells was less than 1%. $CD7^+$ and $CD7^-$ cells were separated by magnetic activated cell sorting (MACS; Miltenyi Biotec GmbH, Bergisch Gladbach, FRG) [10]. Briefly, cells were labeled with monoclonal anti-CD7 antibody (3A1; Biozol, Ech-

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Abbreviations: AD, atopic dermatitis; CLA, cutaneous lymphocyte antigen; SIL, skin-infiltrating lymphocytes.

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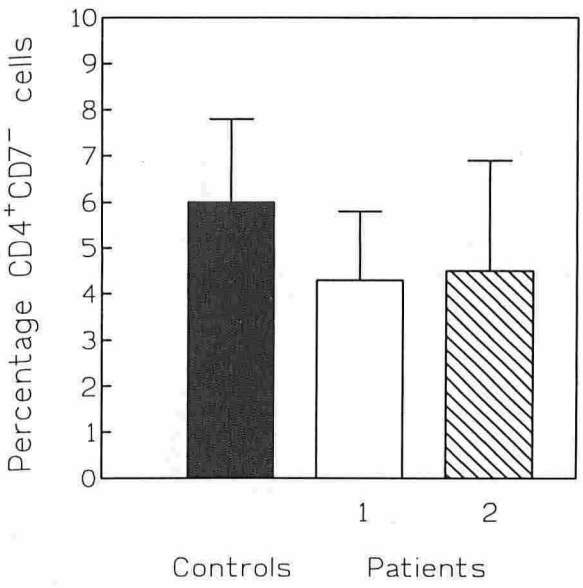

Figure 1. Phenotypic identification of a $CD4^+CD7^-$ T-cell subset in the peripheral blood of patients with psoriasis vulgaris (n = 7; *open bar*), atopic dermatitis (n = 24; *striped bar*), and age-matched healthy controls (n = 39; *solid bar*). Two-color immunofluorescence analysis was performed simultaneously using FITC- and PE-conjugated monoclonal antibodies, as described in *Materials and Methods*. Data are expressed as mean percentage $CD4^+CD7^-$ cells ± SD.

ing, FRG) for 30 min at 4°C, washed in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) and incubated with biotinylated goat anti-mouse immunoglobulin (Ig)M + IgG $F(ab')_2$-fragments (Dianova, Hamburg, FRG). Subsequently, the cells were incubated with fluorescein isothiocyanate (FITC)- conjugated avidin (Becton Dickinson) and with colloidal superparamagnetic microbeads with functional biotin groups (Miltenyi Biotec GmbH). Labeled ($CD7^+$) cells were separated from unlabeled ($CD7^-$) cells by passage through a steelwool column placed into a magnetic field of 0.6 Tesla. Under these conditions, $CD7^-$ cells passed the column whereas $CD7^+$ cells adhered to the steelwool. $CD7^+$ cells could be recovered by removal of the column from the magnetic field and elution with PBS. Homogeneity of purified $CD7^-$ cells (<3% positive cells) and $CD7^+$ cells (>97% positive cells) was monitored by immunofluorescence analysis.

Immunophenotyping Flow-cytometric analyses were performed by two- and three-color immunofluorescence using FITC-, phycoerythrin (PE)-, and peridinium chlorophyll protein reagent (PerCP)-conjugated monoclonal antibodies (MoAb). For indirect immunofluorescence, cells were labeled with unconjugated MoAb followed by PE-conjugated rabbit anti-mouse immunoglobulin $F(ab')_2$ fragments (Dakopatts, Hamburg, FRG).

Fluorescence was excited at 488 nm with a 15-mW Argon laser on a fluorescence-activated cell sorter (FACS) (FACScan; Becton Dickinson, Mountain View, CA). Simultaneous forward and side-scatter analyses were used to gate the lymphocyte population. Fluorescence intensities of 5000 cells/probe were determined.

The following antibodies were used: T-cell receptor (TCR)-1 (alpha/beta-TCR), Leu-5 (CD2), Leu-4 (CD3), Leu-3a (CD4), Leu-2a (CD8), Leu-11 (CD16), Leu-18 (CD45RA), Leu-7 (CD57), IL-2-Rec (CD25), all from Becton Dickinson; UCHL1 (CD45R0), from Dakopatts, Glostrup, Denmark; intercellular adhesion molecule-1 (ICAM-1; CD54), from Dianova, Hamburg, FRG; CD27, CD28, from Janssen Biochimica, Brüggen-Bracht, FRG; 3A1 (CD7) from Biozol, Eching, FRG; HECA-452 (cutaneous lymphocyte antigen CLA, kindly provided by Dr. Louis Picker, Southwestern Medical Center, Dallas, Texas).

Cloning Skin-infiltrating lymphocytes isolated from a psoriasis skin lesion were cloned in microtest plates (Nunc, Roskilde, Denmark) at 0.2–0.3 cells/well in RPMI 1640 medium containing 10% human serum, IL-2 (100 U/ml), OKT3 (2.5 ng/ml), and pooled PBMCs. Growing cells were propagated for more than 4 months by subculturing every 3–4 d.

Table I. Phenotypic Analyses of SILs[a]

Patient	Diagnosis	$CD4^+$	$CD8^+$	$CD4^+CD7^+$	$CD4^+CD7^-$
1	Psoriasis	79	6	2	77
2	Psoriasis	83	8	58	25
3	Psoriasis	89	9	45	44
4	Psoriasis	65	27	52	13
5	Psoriasis	91	9	69	22
6	Psoriasis	90	12	53	37
7	Psoriasis	92	7	56	36
8	AD	94	<2	68	26
9	AD	78	17	65	13
10	AD	87	20	35	52
11	AD	74	24	54	20
12	AD	97	3	53	44
13	CTCL	75	15	50	25
14	CTCL	78	11	67	11
15	CTCL	92	4	84	8
16	CTCL	25	75	19	6
17	Lichen planus	71	14	45	26
18	Lichen planus	35	63	19	16
19	PRP[b]	89	6	75	14
20	PLA[c]	26	46	14	12

[a] Data are expressed as percent positive cells.
[b] Pityriasis rubra pilaris.
[c] Pityriasis lichenoides acuta.

Cell Activation Purified normal $CD7^-$ and $CD7^+$ cells, expanded SILs, and propagated skin-derived T-cell clones were incubated in RPMI 1640 medium, 10% human serum, in the presence of 5 μg/ml PHA (Biochrom), 400 ng/ml ionomycin (Calbiochem, Frankfurt, FRG), 10 ng/ml 12-O-tetradecanoyl-phorbol-13-acetate (TPA) (Sigma) or combinations of these for different periods of time (6 h, 12 h, 24 h, 48 h, 72 h, 96 h). Subsequently, 5×10^5 cells were harvested and analyzed for CD4 and CD7 surface antigen expression by flowcytometry.

Northern Blot Analysis Total cellular RNA was isolated from cells by lysis in a solution containing 4 mM guanidinium isothiocyanate, 50 mM Tris, pH 7.6, 10 mM ethylenediamine tetraacetic acid (EDTA), 10 mM beta-mercaptoethanol, 2% sodium dodecyl sulfate (SDS), and sedimentation through a cushion of 5.7 M CsCl, 100 mM EDTA, pH 7.5, by centrifugation at 200,000 × g for 12 h. RNA was extracted with chloroform and butanol, and precipitated with ethanol. RNA was transfered by blotting techniques onto Hybond N membranes (Amersham Buchler, Braunschweig, FRG). For hybridizations, the following cloned DNA fragments were isolated from vector DNA sequences before radioactive labeling: 1.7 kilobase (kb) CD7 cDNA [11], 1.2 kb PstI fragment of pGAP dehydrogenase [12].

Table II. Surface Phenotype of Individual T-Cell Clones[a]

	Clone			
Antigen	Pso3	Pso13	Pso30	Pso33
TCR-1	>98	>98	>98	>98
CD2	>98	>98	>98	>98
CD3	>98	>98	>98	>98
CD4	>98	>98	>98	>98
CD7	<2	<2	97	>98
CD8	<2	<2	<2	<2
CD16	<2	<2	<2	<2
CD27	<2	<2	<2	<2
CD28	54	39	53	31
CD45RO	>98	>98	>98	>98
CD45RA	<2	<2	<2	<2
CD54	4	10	6	<2
CD57	4	<2	<2	4
Leu-8	<2	<2	<2	<2
HECA-452	58	27	27	15

[a] T-cell clones were established from a psoriasis skin lesion and propagated in the presence of IL-2. Surface marker phenotypes were established by flow cytometry. The values represent percent positive cells.

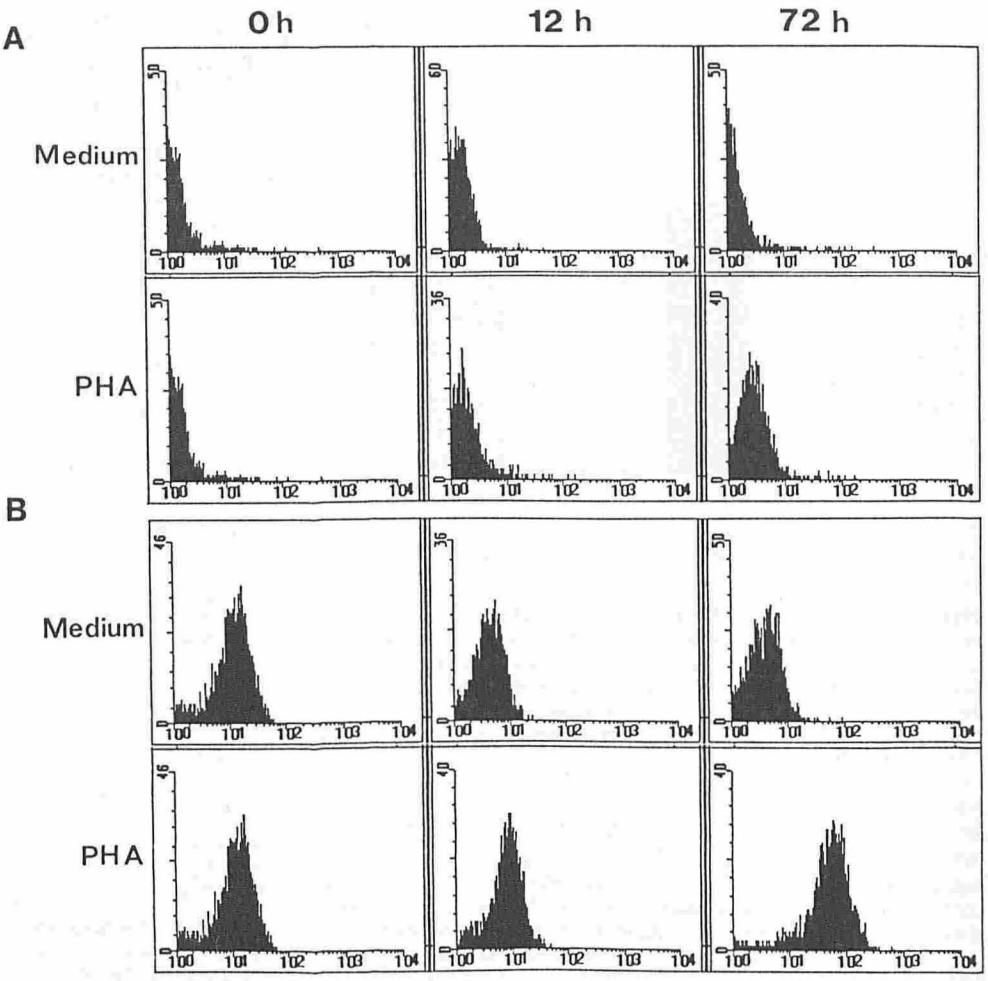

Figure 2. Analysis of CD7 regulation in $CD7^-$ *(A)* and $CD7^+$ *(B)* T-cell clones derived from a psoriatic skin lesion. Clones were stimulated with PHA (5 μg/ml) *in vitro* and expression of CD7 was monitored by flow cytometry 12 and 72 h after stimulation. For control reasons, cells of each clone were cultured in medium and subsequently analyzed by flow cytometry. Data are expressed as single histograms and show expression of CD7 as *red fluorescence.*

Statistical Analyses Differences between data sets were assessed using the Mann-Whitney U-test. Data are expressed as mean ± standard deviation (SD). Coefficient of correlation was determined by linear regression.

RESULTS

Identification of $CD7^-$ Helper T Cells in the Peripheral Blood of Normal Humans and Patients with Inflammatory Skin Diseases $CD4^+CD7^-$ T cells represent 9 ± 3.4% of circulating PBMCs in normal healthy donors (unmatched, n = 39; age range, 1–86 years). Interestingly, the number of circulating $CD4^+CD7^-$ T cells correlates with the age of the donors ($r = 0.62$; $p < 0.01$). Three-color immunofluorescence analysis showed that CLA antigen is expressed on an average of 58% of $CD4^+CD7^-$ T cells (range, 42–76%, n = 10), but only on an average of 9% of $CD4^+CD7^+$ T cells (range, 6–12%, n = 10). Furthermore, we determined the number of circulating T cells with the $CD4^+CD7^-$ phenotype in benign inflammatory skin diseases by immunofluorescence analyses (Fig 1). By mean, 4.1 ± 1.1% of circulating PBMCs from psoriasis patients (n = 7; mean age, 25.3 years; range, 21–31 years) and 4.5 ± 2.4% of PBMCs from atopic dermatitis patients (n = 24; mean age, 24.5 years; range, 20–29 years) express the $CD4^+CD7^-$ phenotype. No difference in the number of $CD4^+CD7^-$ T cells between age-matched healthy controls (n = 25; mean age, 26.5 years; range, 20–30 years) compared to patients with inflammatory dermatoses was recorded.

Isolation and Immunophenotyping of SILs Lymphocyte cultures were generated from punch biopsies (n = 20) of various inflammatory skin lesions. After 8–12 d of culture, cells that had migrated out of tissue were harvested and analyzed by flow cytometry. SILs contained a high portion of $CD7^-$ T cells (range, 8–75%) in comparison to those of the peripheral blood ($p < 0.01$). All cells expanded expressed the CD3 antigen and the majority of $CD7^-$ SILs expressed the $CD4^+$ helper T cell phenotype (Table I). The predominance of $CD7^-$ cells in propagated SIL cultures is unlikely to be a culture artefact because cultivation of PBMCs under identical conditions revealed the same portion of $CD7^-$ and $CD7^+$ T cells as monitored in peripheral blood (not shown). We established three $CD7^-$ T-cell clones derived from SILs of a psoriatic skin lesion. For control reasons two $CD7^+$ clones from the same biopsy were generated and propagated under identical culture conditions. During a culture period of more than 4 months, all clones retained their pattern of CD7 antigen expression. Furthermore, immunophenotypic analyses of various T-cell markers on propagated clones were performed. All of the $CD7^-$ clones expressed CD4 antigen and alpha/beta TCR to the same extent as $CD7^+$ clones. No clones were observed that express CD45RA, whereas all clones were positive for CD45RO. All clones contained cells that expressed CLA antigen although the number of cells stained in each of the clones differed widely. Furthermore, all clones had a comparable high expression of major histocompatibility complex (MHC) class II antigens and the p55-IL-2 receptor (not shown). In summary, all clones showed the same pattern of surface antigen expression except for CD7 (Table II).

Modulation of CD7 Antigen Expression *In Vitro* To analyze whether CD7 expression can be induced in $CD7^-$ T cells, SILs were stimulated with PHA or Io/TPA *in vitro* and analyzed by flow-cyto-

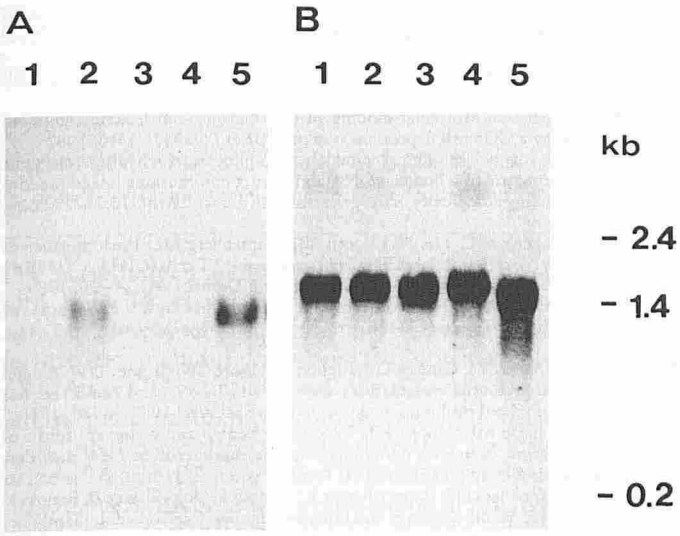

Figure 3. Total cellular RNA (15 μg) of CD7⁻ T-cell clones *(lanes 1, 3, 4)* and of CD7⁺ T-cell clones *(lanes 2, 5)* isolated from a psoriasis skin lesion were hybridized to the 1.7-kb CD7 cDNA probe *(A)*. For control reasons, the Northern blot was rehybridized to the 1.2-kb PstI fragment of pGAPD DNA *(B)*.

metry. These agents were not capable of inducing CD7 expression in CD7⁻ cells, whereas CD7 antigen expression increased in CD7⁺ cells. For comparison, CD4 expression on both CD7⁺ and CD7⁻ T cells, respectively, decreased to the same degree after stimulation *in vitro*.

Furthermore, CD7⁻ and CD7⁺ T-cell clones derived from psoriatic SILs were stimulated using PHA or Io/TPA *in vitro*. As expected, CD4 expression decreased within 48 h in all clones tested. Moreover, CD7 expression increased on CD7⁺ clones, whereas CD7⁻ T-cell clones did not express detectable CD7 antigen even after stimulation (Fig 2). On the other hand, CD4 expression decreased in all clones after incubation with ionomycin plus TPA, with minimal expression 48 h after stimulation. The CD7⁺ and CD7⁻ subpopulations from the peripheral blood, respectively, showed the same response after stimulation with PHA or Io/TPA (not shown).

Analysis of CD7 mRNA Transcripts CD7⁻ T-cell clones isolated from a psoriatic skin lesion and propagated *in vitro* in the presence of IL-2 were subjected to Northern blot analysis for CD7 mRNA expression. As shown in Fig 3, no CD7-specific mRNA transcript could be detected whereas cells of the CD7⁺ clones express the 1.7-kb CD7 mRNA, as expected.

DISCUSSION

Recently, we characterized a small subset of peripheral T cells that lack expression of the CD7 antigen [8]. These cells represent a physiologic subpopulation of normal human T cells from peripheral blood. The majority of circulating CD7⁻ T cells express the CD4⁺CD45RO⁺CD45RA⁻ memory phenotype of helper T cells. Molecular analyses have revealed a regulation of CD7 expression at the level of mRNA transcription. These data suggest that the lack of CD7 characterizes a particular subset of memory helper cells in the peripheral blood of normal individuals.

The mononuclear cell infiltrates of most inflammatory skin diseases have been found to be predominantly CD4⁺ helper T cells [13]. Moreover, the majority of lesional helper T cells in inflammatory dermatoses, e.g., atopic dermatitis, psoriasis, nummular dermatitis, and pityriasis rosea, were reported to express the memory helper phenotype [14]. In addition to benign cutaneous infiltrates, the predominance of the CD4⁺ memory helper phenotype has been demonstrated in skin lesions of CTCL. A high number of lesional T cells in different types of CTCL were shown to lack CD7 expression, in contrast to the peripheral blood [5,6]. This phenomenon has been reported to be helpful in differentiating a malignant lymphoma from reactive cutaneous infiltrates. Furthermore, nonexpression of CD7 is a classical feature of leukemic Sézary cells in CTCL [7]. However, recent immunohistologic studies indicated that lack of CD7 expression on T cells is not confined to malignant lymphoid infiltrates but can be also found in benign inflammatory skin lesions† [15,16]. In addition, a high number of CD7⁻ T cells was found in normal epidermal and dermal skin [17,18]. To elucidate whether skin-associated T lymphocytes are of CD7⁻ phenotype, we analyzed SILs derived from various inflammatory skin diseases in comparison to peripheral blood lymphocytes. Unexpectedly, SILs isolated from different benign and malignant skin lesions contained a high portion of CD7⁻ memory helper T cells, whereas the portion of these cells in the peripheral blood was not altered compared to those of normal healthy controls. Cell activation *in vitro* upregulated CD7 expression in positive cells but did not induce CD7 expression in negative T cells. These results confirm our recent findings obtained with fresh T cells from normal healthy donors [8]. Thus, absence of CD7 seems to be a stable phenotype in a major subset of SILs. Analysis of long-term cultured CD7⁻ helper T-cell clones derived from psoriatic skin lesions revealed no phenotypic change in both CD7⁻ and CD7⁺ clones with respect to CD7 expression. Northern blot analysis of psoriatic CD7⁻ clones indicated absence of CD7 at the mRNA level. Our data presented here support evidences for a separate lineage in the late phase of T-cell differentiation. We speculate that the subset of T cells found in normal skin and certain inflammatory skin lesions may be identical to or derived from CD7⁻ T cells of the peripheral blood, which may display skin tropism. Consistent with recent reports, we conclude that CD7 expression on cutaneous infiltrates is not a good marker to differentiate benign inflammatory reactions from malignant infiltrates [15].

The physiologic role of CD7 remains to be determined. CD7 MoAb crosslinking was reported to induce changes in intracellular Ca^{2+}, as well as stimulation of T-cell proliferation [19]. Therefore, CD7⁻ has been discussed to play a role in modulating T-cell–adhesive interactions. However, the ligand for CD7 remains to be identified. On the other hand, absence of CD7 expression on T cells in normal individuals characterizes a particular subset of memory cells and is not a marker for T-cell activation. We monitored that absence of CD7 is associated with a weak proliferation in response to mitogens and anti-CD3 MoAb, in contrast to CD7⁺ cells [8]. Recent results indicate that CD7⁻ T cells may show a more pronounced proliferative response when stimulated *via* the CD2 pathway [20]. Thus, absence of CD7 on T cells might correspond to a subset of memory cells with a different activation pathway. Recent studies indicate that the majority of T cells infiltrating cutaneous sites of inflammation express a carbohydrate ligand (CLA) for ELAM-1, which appears to act as a skin lymphocyte homing receptor [21]. In addition to cutaneous infiltrates CLA was reported to be expressed on a minor subpopulation of circulating memory cells in normal individuals [22]. Interestingly, our studies demonstrate that a major subset of normal circulating CD7⁻ T cells express CLA antigens. We suggest that functional analyses of this particular T-cell subset might generate more information about the physiologic role of CD7⁻ T cells in the human skin. Moreover, the exact identification of different stages in late-phase T-cell maturation

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might help to clarify whether $CD7^-$ cells may represent the physiologic counterpart of malignant Sézary cells.

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