

CD4⁺CD7⁻CD57⁺ T Cells: A New T-Lymphocyte Subset Expanded During Human Immunodeficiency Virus Infection

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CD7 and CD57 are two cell surface molecules related to the differentiation or functional stages of CD4⁺ T cells. The CD4⁺CD7⁻ T cells represent a minor subset of CD4⁺ cells in normal individuals and are considered to contain the normal counterpart of S ezary T cells; the CD4⁺CD57⁺ peripheral blood lymphocytes (PBL) are detectable in long-term renal allograft recipients. We compared the cell surface expression of these CD7 and CD57 markers on CD4⁺ T lymphocytes in peripheral blood and lymphoid organs from normal individuals and human immunodeficiency virus (HIV)-infected patients. Our results indicate that CD4⁺CD7⁻ T cells in normal PBL do not express CD57 and were poorly responsive to anti-CD3 monoclonal antibody (MoAb), the activation being

restored by addition of anti-CD28 MoAb. This CD4⁺CD7⁻ cell subset is increased in peripheral blood during HIV infection, and its progressive expansion mirrors both the absolute and relative decrease of CD4⁺ T cells. The lack of CD7 expression is correlated with CD57 acquisition on CD4⁺ T cells because CD4⁺CD7⁻CD57⁺ cells represent a major component of the CD4⁺CD7⁻ subset in HIV-infected patients. Our results suggest that the presence and the expansion of CD4⁺CD7⁻CD57⁺ T lymphocytes, which do not behave as previously defined helper subsets, may participate to the immune dysfunction observed during HIV infection.

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THE CD4⁺ HELPER cells are commonly subdivided into naive cells (CD45RA⁺) and memory helper cells (CDw29⁺, CD45RO⁺). Indeed, these cells differ by their function because virgin CD4⁺ T cells weakly proliferate when stimulated via CD2 and CD3.¹⁻³ In addition to these markers, CD4⁺ cells may also express or lose molecules characterizing minor subsets expanded in some pathologic situations. Among the markers that could be considered for such matter are the CD7 and CD57 antigens.^{4,5}

The CD7 antigen is the earliest T-cell differentiation antigen, detectable on bone marrow T-cell precursors.⁶ A small subset of peripheral CD4⁺ T cells can lack CD7 surface expression in normal individuals^{1,5,7} and the nonexpression of CD7 is a classical feature on malignant CD4⁺ S ezary T cells.^{5,7-9} The CD7 molecule is involved in T-cell activation^{6,10,11} and is supposed to be the T-cell receptor for IgM.¹⁰

On the other hand, the CD57 antigen, related to neural adhesion molecules,¹² has been initially described on large granular lymphocytes, CD8 suppressor T lymphocytes,^{13,14} and has been reported in a small subset of CD4⁺ T cells in tonsils from normal individuals.¹⁵ More recently, the CD57 molecule has been detected on an important subset of peripheral CD4⁺ T lymphocytes from renal allograft recipients⁴ that does not behave as previously defined helper CD4⁺ lymphocytes.

The depletion of CD4⁺ T lymphocytes is considered as a major hallmark of the cellular immune deficiency observed

in human immunodeficiency virus (HIV)-infected patients.¹⁶ CD4⁺ T cell decrease does not occur at random because, in asymptomatic HIV-infected patients, memory T cells are preferentially lost, resulting in an early decrease in T-cell functions.¹⁷ Indeed, the low responsiveness of CD4⁺ T lymphocytes to anti-CD3 stimulation observed in HIV-infected patients might be highly predictive for progression toward acquired immunodeficiency syndrome (AIDS).¹⁸ However, the characterization of CD4⁺ cell surface phenotype in HIV-infected patients remains limited to some markers. The combined presence of CD7 and CD57 antigens has not been studied up to now on CD4⁺ T cells in HIV infection. To document such phenotypes, we have investigated the CD7 and CD57 expression on CD4⁺ T lymphocytes in a large series of HIV-infected patients and in a panel of seronegative controls. Our data show that the lack of CD7 identifies a normal CD4⁺ memory T-cell subset poorly responsive when stimulated via CD3. We reported a relative increase of the CD4⁺ lymphocytes that lack CD7 and coexpress CD57 in HIV patients. More importantly, these cells are correlated with prognostic markers and could be partly responsible for the qualitative immune dysfunction observed in AIDS.

MATERIALS AND METHODS

Patients. We studied 78 seropositive HIV-infected patients observed in the Department of Infectious Diseases of the Piti -Salp etri re Hospital. The seropositivity was assessed by enzyme-linked immunosorbent assay (ELISA) and Western blotting (ELAVIA and New LAV Blot I; Diagnostics Pasteur, Marnes-La-Coquette, France). Data from HIV patients were compared with 43 seronegative controls: normal blood samples from 32 healthy volunteers, bone marrow samples from three normal donors for allogeneic bone marrow transplantation, thymus samples from two children undergoing cardiac surgery, three tonsil samples from laryngologic surgery, and three spleen samples from posttraumatic abdominal surgery.

Monoclonal antibodies (MoAbs). A panel of 28 murine MoAbs was used in this study. Anti-CD4 (IOT4), anti-CD7 (IOT7), anti-CD11a (IOT16), anti-CD16 (ION16), anti-CD18 (IOT18), anti-CD23 (IOB8), anti-CD45RA (IOL2), and anti-CD54 (IOM54) were purchased from Immunotech (Marseille-Luminy, France). Anti-CD34 (MY10), anti-CD38 (Leu17), anti-CD57 (Leu7), and anti-TCR α/β were obtained from Becton-Dickinson (Grenoble, France). We purchased from Coultronics (Margency, France)

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anti-CD1a (T6), anti-CD2 (T11), anti-CD3 (T3), anti-CD5 (T1), anti-CD7 (3A1), anti-CD8 (T8), anti-CD10 (J5), anti-CD14 (MY4), anti-CD19 (B4), anti-CD25 (IL2R1), anti-CD26 (Ta1), anti-CDw29 (4B4), anti-CD56 (NKH1), anti-CD71 (T9), anti-HLA-DR, TQ1 (LAM1, not clustered).

Cells. Peripheral mononuclear cells were isolated using Ficoll Hypaque gradient (Pharmacia, Uppsala, Sweden). Normal lymphoid organs were teased out immediately after ablation and mononuclear cells separated as described above.

Separation procedure. Purified lymphocyte subsets were sorted using panning techniques completed by immunomagnetic separation. Before purification, monocytes were removed by incubating cells in RPMI 1640 medium in culture flasks at 37°C and 5% CO₂ for 1 hour. Nonadherent cells were collected, washed, and suspended in RPMI 1640. CD7⁺ T cells were removed using panning techniques. Culture flasks were coated with anti-CD7 I21 (a kind gift of A. Bernard, Institut Gustave-Roussy, Villejuif, France) for 16 hours at 4°C in RPMI 1640. After washing, nonadherent mononuclear cells were incubated in these culture flasks for 90 minutes in RPMI 1640 at 4°C. The supernatant was collected and washed, and cells were counted. In a third step, a cocktail of I21 (CD7) and T8 (CD8) MoAbs was added to residual cells and magnetic microbeads coated with antimouse IgG (Dynal, Oslo, Norway) used (as directed by manufacturer) to remove labeled cells. To further purify CD4⁺CD7⁻ T lymphocytes, cells were then incubated with a cocktail of MY4 (CD14), ION16 (CD16), and B4 (CD19) MoAbs and labeled cells removed by the same magnetic beads. Cell purity was controlled by two distinct epitopes of CD7 (3A1-phycoerythrin [3A1-PE], IOT7-fluorescein isothiocyanate [IOT7-FITC]) and exceeded 98% in each experiment.

Immunomagnetic separation was also used to purify CD4⁺CD7⁺ T lymphocytes (TL) from nonadherent mononuclear cells: depletion with anti-CD8, CD14, CD16, and CD19 MoAbs was performed as previously described. After each experiment, viability of the cells was controlled by Trypan Blue exclusion (up to 95% viable cells).

Immunofluorescence analysis. Mononuclear or purified cells (1.10⁶ cells) in a total volume of 0.1 mL were incubated for 20 minutes at 4°C in the dark with FITC and PE-MoAb for two-color immunofluorescence staining. For three-color staining, cells were first incubated with a biotin-conjugated MoAb and then with a streptavidin-PE-Texas-Red complex (Duochrom; Becton-Dickinson, Mountain View, CA). In the last step, FITC and PE-MoAb were added to the cells. After each step, cells were washed with 2 mL of phosphate-buffered saline (PBS)-0.5% bovine serum albumin (BsA) and centrifuged. Controls consisted in cells stained with irrelevant mouse MoAb directly conjugated to FITC, PE, and biotin to assess Fc-related nonspecific binding.

For intracytoplasmic staining, the cells were permeabilized with the saponin technique.

Samples were analyzed on a FACSCAN flow cytometer (Becton-Dickinson, Sunnyvale, CA). Peripheral blood mononuclear cells were characterized by two- or three-color fluorescence staining. The lymphoid cells were first gated by their physical properties (forward and right angle scattering). Then, only CD4⁺ lymphocytes were gated by fluorescence emission and analyzed for at less 5,000 cells.

Proliferation assays. Proliferation assays used various combinations of stimulating agents: immobilized anti-CD3 MoAb UCHT1, 1/200 (P.J.C. Beverley, Cancer Research Institute, London, UK); anti-CD28 MoAb 248-23, 1/100 (D. Olive, Marseille-Luminy, France); mitogenic combination of anti-CD2 MoAbs X11 and D66, 1/100, (A. Bernard, Institut Gustave-Roussy, Villejuif, France); phytohemagglutinin (PHA-M), 10 µg/mL and phorbol myristate acetate (PMA), 1 ng/mL. Controls consisted of supplemented medium alone. Purified T cells (0.5.10⁵ cells) were incubated in triplicate in 96-well flat-bottom plates in a total volume of 0.2 mL RPMI 1640 supplemented with 10% (vol/vol) fetal calf serum, 1% glutamine, 1% pyruvate, 1% nonessential amino acids (MEM). Cultures were pulsed overnight with 1 µCi (³H)-thymidine and were harvested subsequently. Incorporated radioactivity was measured at day 2 and day 5 by liquid scintillation counting (LS1701 counter; Beckman, Gagny, France).

Statistical tests. Statistical analysis used correlation tests and *t*-tests.

RESULTS

Phenotypic characterization of CD4⁺CD7⁻ T lymphocytes in normal individuals. To compare the CD7 expression on CD4⁺ T cells from HIV-infected patients and control subjects, we first studied the CD4⁺CD7⁻TL population in peripheral blood and in lymphoid organ samples from 43 seronegative individuals. The CD4⁺ T lymphocytes that do not express CD7 cell surface antigen were detectable as shown in Table 1. They represented a minor subset of the CD4⁺ PBL (10% ± 4%). The same distribution was observed in lymphoid organs: spleen (9% ± 3%), tonsil (10% ± 3%), thymus (9% ± 2%), bone marrow (13% ± 1%).

Cell surface expression of CD7 was controlled with two anti-CD7 MoAbs (3A1, IOT7). Both MoAbs gave similar results although higher fluorescence intensity could be obtained with 3A1-PE. This lack of CD7 cell surface expression could be related to a downmodulation. How-

Table 1. CD7 and CD57 Expression of CD4 Lymphocytes of Seronegative Controls

		PBL	Spleen	Bone Marrow	Thymus	Tonsil
CD4 ⁺ CD7 ⁻ TL	N*	32	3	3	2	3
	%†	10 ± 4	9 ± 3	13 ± 3	9 ± 2	10 ± 3
CD4 ⁺ CD57 ⁺ TL	N	10	3	3	2	3
	%	2 ± 1	2 ± 1	3 ± 1	2 ± 1	13 ± 6
CD4 ⁺ CD57 ⁺ CD7 ⁺ ‡	%	1 ± 1	2 ± 1	ND§	ND	10 ± 6
CD4 ⁺ CD57 ⁺ CD7 ⁻	%	0.3 ± 0.3	1 ± 1	ND	ND	3 ± 1

*Number of samples studied.

†Percentage of positive cells (mean ± SD).

‡Subsets identified by three-color staining.

§Not done.

Table 2. Phenotypic Characterization of Normal Purified CD4⁺CD7⁻ T Cells

CD*	% Cells†	CD	% Cells
2	97 ± 2	25	5 ± 3
3	98 ± 2	26	4 ± 2
4	99 ± 1	29	87 ± 10
5	96 ± 5	34	3 ± 2
7	<1	38	2 ± 2
8	<1	45RA	2 ± 1
10	3 ± 2	56	2 ± 1
11a	95 ± 2	57	5 ± 4
14	1 ± 1	71	2 ± 1
16	2 ± 1	TCR α/β	93 ± 4
18	96 ± 2	HLA-DR	7 ± 4
23	4 ± 3	TQ1	41 ± 10

*Cluster of differentiation or antigen if not clustered.

†Percentage of labeled cells by the MoAb expressed as mean percentage ± standard deviation in four experiments.

ever, we failed to detect any intra-cytoplasmic CD7 expression in the CD4⁺CD7⁻ TL (data not shown). In addition, several modulation experiments were performed. Normal PBL were incubated in RPMI 1640 medium at 4°C, 20°C, and 37°C and the CD7 reactivity controlled after 1, 2, 6, and 18 hours. Results showed that the lack of CD7 cell surface expression was not related to CD7 modulation (data not shown). Three-color staining on peripheral blood cells and double-labeling experiments on purified cells were performed. The CD4⁺CD7⁻ cells did not display monocytic markers (CD14⁻, HLA-DR⁻) but rather were T lymphocytes expressing: CD2, CD5, CD3, TCR α/β. This T-cell subset expressed high levels of CDw29, LAM1 molecule and did not express CD45RA, as previously described.⁵ The CD4⁺CD7⁻ TL were neither activated T cells, as shown by the lack of expression of the CD25, CD26, CD38, CD71, HLA-DR antigens, nor natural killer (NK) cells (CD16⁻, CD56⁻, CD57⁻). In addition, these cells expressed CD11a/

CD18 complex and were negative for CD10, CD23, CD34, and CD54. The results are listed in Table 2. Altogether, these data indicate that CD4⁺CD7⁻ lymphocytes are mature T cells in normal individuals.

Functional analysis of CD4⁺CD7⁻ TL in normal individuals. The proliferative responses of purified CD4⁺CD7⁻ TL were compared with purified CD4⁺CD7⁺ TL responses. These cells were obtained by panning methods resulting in depletion of accessory cells. We used a panel of seven stimulating reagents including: mitogenic combination of anti-CD2 MoAbs, immobilized anti-CD3 MoAb, anti-CD28 MoAb, anti-CD3 + PMA, anti-CD3 + anti-CD2 MoAbs, anti-CD3 + anti-CD28, PMA + PHA. The results of one typical experiment out of five are detailed in Table 3. The overall absolute CD4⁺CD7⁻ TL proliferative responses obtained at day 2 were consistently lower than CD4⁺CD7⁺ TL ones, although the stimulation indexes were comparable. Results differed at day 5: CD4⁺CD7⁻ TL had normal proliferative responses when stimulated via CD2, contrasting with weak responses obtained with anti-CD3 MoAb even if associated with PMA. Similarly, the PHA + PMA responses remained low. In contrast, the addition of anti-CD28 MoAb restored the CD3-induced proliferation.

Altogether these data point out the presence of a normal minor CD4⁺ T-cell subset particular by its lack of CD7 expression and by its dissociated responses when stimulated via CD3 or CD2.

Analysis of the CD4⁺CD57⁺ T lymphocytes in normal individuals. In addition to CD7, we investigated the CD57 expression on the CD4⁺ T lymphocytes. Figure 1 shows typical results obtained with anti-CD4, anti-CD7, and anti-CD57 MoAbs on purified normal CD4⁺CD7⁻ TL and normal CD4⁺ peripheral lymphocytes. We studied normal peripheral blood and the same panel of normal lymphoid organs. When analyzing the CD57 fluorescence on gated CD4⁺ T lymphocytes, we individualized CD4⁺CD57⁺ cells.

Table 3. Proliferative Responses of Purified CD4⁺/CD7⁻ and CD4⁺/CD7⁺ T Lymphocytes

Cell Treatment	CD4 ⁺ /CD7 ⁻ TL		CD4 ⁺ /CD7 ⁺ TL	
	Day 2	Day 5	Day 2	Day 5
Medium	140 ± 50*	266 ± 69	1,024 ± 181	2,700 ± 800
Anti-CD3†	616 ± 115	1,100 ± 360	2,900 ± 1,000	85,798 ± 6,287
	4.4‡	4.1	2.8	32
Anti-CD2§	6,513 ± 400	24,745 ± 5,180	13,790 ± 2,230	76,215 ± 3,633
	47	93	14	28
Anti-CD28	1,190 ± 294	346 ± 14	2,000 ± 783	2,700 ± 1,000
	8.5	1.3	1.9	1
Anti-CD3 + CD28	5,079 ± 656	91,823 ± 15,120	18,250 ± 3,900	242,387 ± 26,244
	36	345	18	90
Anti-CD3 + CD2	4,748 ± 502	93,310 ± 15,030	30,344 ± 1,447	66,000 ± 8,376
	34	350	30	24
Anti-CD3 + PMA	1,178 ± 149	2,006 ± 270	14,232 ± 2,010	162,798 ± 28,391
	8.4	7.5	14	61
PHA + PMA	1,762 ± 360	8,992 ± 1,160	16,820 ± 1,928	28,956 ± 7,690
	13	34	16	11

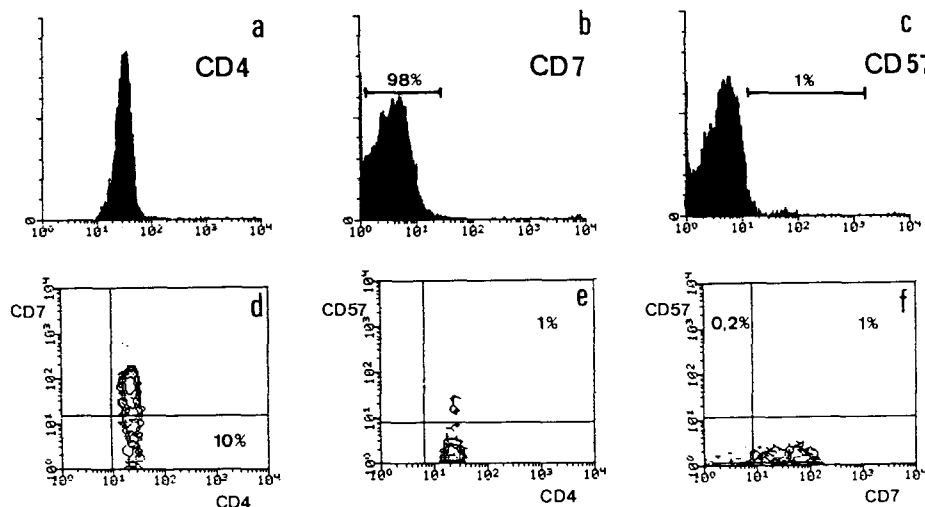
*cpm expressed as mean ± SD.

†Immobilized anti-CD3 MoAb.

‡Stimulation index = experimental cpm/spontaneous cpm.

§Comitogenic combination of X11 and D66 MoAbs.

Fig 1. CD4, CD7, and CD57 expression in seronegative controls. Histograms a through c: Double-labeling experiment on purified CD4⁺CD7⁻ T lymphocytes; cursors on histograms b and c underline the percentages of CD7⁻ (histogram b) and CD57⁺ cells (histogram c). Dot plots d through f: Three-color staining on CD4⁺ gated peripheral lymphocytes illustrates the presence of 10% of CD4⁺CD7⁻ T lymphocytes (dot plot d); as mentioned in the Results, no CD4⁺CD7⁻CD57⁺ T lymphocytes could be detected (dot plot f). Cursors show background staining.



These cells were a minor subset of CD4⁺ T cells in peripheral blood ($2\% \pm 1\%$), thymus ($2\% \pm 1\%$), spleen ($2\% \pm 1\%$), and bone marrow ($3\% \pm 1\%$). As already reported,¹⁵ only tonsil CD4 lymphocytes expressed significant levels of CD57 ($13\% \pm 6\%$, $P < .001$ v other organs). A three-color immunofluorescence analysis was performed on the same controls. Results (Table 1) showed that CD57 was mainly expressed on CD4⁺CD7⁺ lymphocytes. No CD4⁺CD7⁻CD57⁺TL could be detected in peripheral blood ($0.3\% \pm 0.31\%$), as mentioned above, but only in tonsils where a very low percentage of CD4⁺CD7⁻CD57⁺TL was detectable. They represented $3\% \pm 1\%$ of tonsil CD4⁺ T cells ($P < .001$ v PBL and other lymphoid organs).

Characterization of the CD4⁺CD7⁻CD57⁺ T-lymphocyte subset in HIV-infected patients. We investigated the CD7 expression on CD4⁺ T lymphocytes in 78 HIV-infected patients. The results differed markedly from normal seronegative controls (Table 4) because the mean percentage of CD4⁺CD7⁻TL was expanded in HIV-infected patients ($33\% \pm 21\%$, $P < .001$). A hydrophobic sequence in the transmembrane region of CD7 shares similarities with fusion sequences in the transmembrane region of HIV-1 gp41.¹⁹ Thus, we studied a possible CD7 modulation induced by anti-HIV antibodies. Normal peripheral CD4⁺ lymphocytes were incubated for 1, 2, 6, 16, and 24 hours with serial dilutions of HIV-infected sera. Then, the mean fluorescence channel of anti-CD7 MoAb and the percentage of CD4⁺CD7⁻TL were analyzed. No induction of CD7-negative cells could be observed (data not shown).

We also observed an expansion of peripheral blood CD4⁺ lymphocytes positive for the CD57 marker (Table 5). These cells represented $14\% \pm 15\%$ of the CD4⁺ population ($P < .001$ v seronegative controls). The CD4⁺CD7⁻ and CD4⁺CD57⁺ subsets were significantly linked as observed in 46 HIV-infected patients (Fig 2a, $r = .90$, $P < .001$). To test whether the CD4⁺CD7⁻ cells were the CD4⁺CD57⁺ cells, we performed a three-color staining in 18 HIV-infected patients and compared it with data obtained from seronegative normal PBL. Figure 3 shows typical results obtained in one HIV-infected patient after staining with anti-CD4, anti-CD7, and anti-CD57 MoAbs. When gating on CD4⁺ T cells, 60% are CD7⁻ (Fig 3d) and 50% are CD57⁺ (Fig 3e). Moreover, 45% of the CD4⁺ cells are both CD7⁻ and CD57⁺ (Fig 3f). Similar data were obtained in the 18 tested patients.

Therefore, in the overall 18 patients, we could subdivide the CD4 lymphocyte population in HIV-infected patients in four subsets. The mean percentages in each subset for the 18 patients were: CD4⁺CD7⁺CD57⁻ cells ($68\% \pm 22\%$), CD4⁺CD7⁺CD57⁺ cells ($3\% \pm 2\%$), CD4⁺CD7⁻CD57⁻ cells ($19\% \pm 12\%$), CD4⁺CD7⁻CD57⁺ cells ($9\% \pm 9\%$). Linear regression analysis (Fig 2b) showed that CD4⁺CD57⁺TL strongly correlated with CD4⁺CD7⁻CD57⁺ cells ($r = .97$, $P < .001$).

These data demonstrate: (1) the expansion of the CD4⁺CD7⁻ subset in the peripheral blood of HIV-infected patients and (2) the expression of the CD57 molecule on CD4⁺CD7⁻ T PBL. Therefore, we can individualize, in

Table 4. Characterization of CD4⁺/CD7⁻ T Lymphocytes in HIV-Infected Patients

	All HIV Patients	CD4 > 400*	CD4 200-400	CD4 < 200
No. of Patients	78	17	23	38
%	33 ± 21 $P < .001$ v NC†	28 ± 17 $P > .05$	23 ± 12 $P < .01$	43 ± 22 $P < .001$
Absolute no. (/mm ³)	67 ± 49	142 ± 87 $P < .001$ v NC	72 ± 51	31 ± 22

*Absolute number of CD4 lymphocytes/mm³.

† P observed v normal seronegative controls.

Table 5. Characterization of CD57 Expression on CD4⁺ T Lymphocytes in HIV-Infected Patients

No. of Patients	All HIV Patients	CD4 > 200*	CD4 < 200
	46	15	15
% CD4 ⁺ CD57 ⁺ TL	14 ± 15	20 ± 21	8 ± 9
	$P < .001$ v NCT†	$P = .04$	

*Absolute number of CD4/mm³.

†P observed v normal seronegative controls.

HIV-infected patients, a new CD4⁺CD7⁻ peripheral T-cell subset that is not detectable in normal PBL and is characterized by the cell surface expression of CD57.

Relationship between the CD4⁺CD7⁻CD57⁺TL subset and disease progression. The CD4⁺CD7⁻CD57⁺ cells were studied according to the percentages and the absolute values of CD4⁺ PBL, considered as a marker of HIV disease progression.

Results of the CD4⁺CD7⁻TL evaluation are listed in Table 4. We subdivided HIV patients (n = 78) into three groups: (1) CD4⁺ cells > 400/mm³, (2) 200 < CD4⁺ cells < 400/mm³, (3) CD4⁺ cells < 200/mm³. The mean percentages of CD4⁺CD7⁻ TL were 43% ± 22%, 23% ± 12%, and 28% ± 17% in groups 3, 2, and 1, respectively. The results from each group differed from those observed in normal PBL ($P < .001$). Among HIV-infected patients, the percentages of CD4⁺CD7⁻TL were significantly increased in patients with CD4 < 200/mm³ if compared with data observed in the other two groups ($P < .001$). Furthermore, regression curve analysis (Fig 4) showed that, in 78 HIV-infected patients, the percentages of CD4⁺CD7⁻TL correlated with the percentages ($r = -.47$, $P < .001$) and the absolute values of CD4⁺ T cells ($r = -.49$, $P < .001$).

In addition, we studied the absolute values of CD4⁺CD7⁻TL in each of the three groups (Table 3). Of note, CD4⁺CD7⁻TL were expanded when CD4 > 400/mm³ ($142 ± 87/mm^3$ v $73 ± 25$ in normal controls, $P < .001$). These data suggest that the expansion of the CD4⁺CD7⁻ subset occurs at early stages of HIV infection but is proportionally increasing along with the depletion of the total CD4⁺ subset.

The correlation between the CD57 expression on CD4⁺ cells and the CD4⁺ cells number was also analyzed in two groups of HIV-infected patients (CD4 < 200, CD4 > 200/mm³). Results showed that the mean percentages observed when CD4 < 200 (20% ± 21%) significantly differed from results in patients with CD4 > 200 (8% ± 9%) and from

normal controls (Table 4). However, the regression analysis showed no correlation between the CD4⁺CD57⁺TL and the decrease of CD4⁺ T cells ($r = -.19$, $P > .10$, data not shown), suggesting different kinetics between the nonexpression of CD7 and the detection of CD57 on CD4⁺ T cells. Finally, eight HIV-infected patients were evaluated to determine whether AZT therapy could influence the levels of CD4⁺CD7⁻CD57⁺ T cells. Chronologic samples were tested: (1) before azidothymidine (AZT) therapy, (2) 2 or 4 months, (3) 6 or 8 months, and (4) 12 or 16 months after AZT introduction. No characteristic variation could be observed for these two cell subsets as well as for the CD8⁺CD57⁺ T lymphocytes subset (data not shown).

Together, the study of the CD7 and CD57 expression on CD4⁺ lymphocytes from HIV-infected patients gave us two important results: (1) the levels of CD4⁺CD7⁻CD57⁺ T cells correlate with the depletion of the total CD4⁺ subset and (2) the CD4⁺CD7⁻ subset is expanded at early stages of the HIV disease.

DISCUSSION

The CD4⁺ T lymphocyte population remains one of the most important criteria to assess cellular immune deficiency in HIV-infected patients. This subset of T cells was already well documented for its expression of CDw29 and CD45 antigens.²⁰ Previous studies suggested that the CD45RO⁺CDw29⁺CD4⁺ subset corresponded to memory CD4⁺ lymphocytes that could be preferentially infected by HIV and, consequently, the first CD4⁺ subset that decreases in peripheral blood.¹⁷ The major goals of our study were to point out the existence of two cell surface markers that could better characterize CD4⁺ lymphocytes during HIV infection, define a new subset abnormally present in such occasions, and provide new prognostic tools.

The CD7 antigen is the first specific marker of T-cell lineage and is expressed during all stages of T-cell differentiation.⁶ However, defective expression of CD7 on CD4⁺ T lymphocytes has already been reported. Indeed, normal CD4⁺ lymphocytes,^{1,5-7} various leukemic T cells^{5,8,9,21} (such as Sézary cells), and synovial CD4⁺ lymphocytes in rheumatoid arthritis²² lacking the CD7 molecule were described. Furthermore, a case of severe combined immunodeficiency with absence of CD7 on T cells has been reported.²³ In Sézary cells, as in our experience on normal T cells, CD4⁺CD7⁻TL exclusively expressed CDw29 and lacked

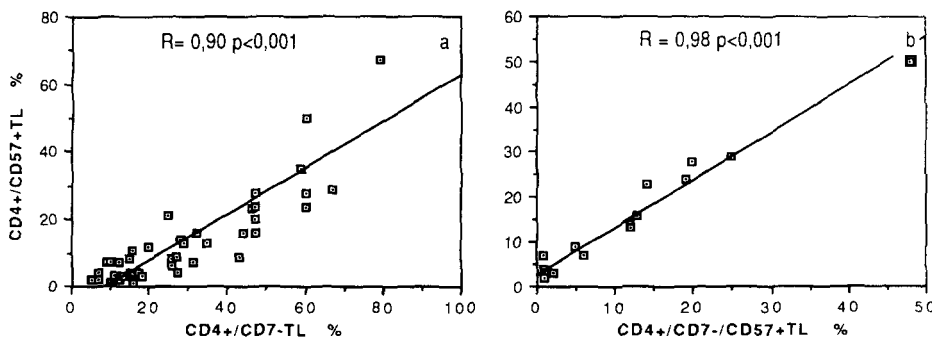


Fig 2. Correlation curves between the percentages of CD4⁺CD57⁺ T lymphocytes (y axis) and (a) the percentages of CD4⁺CD7⁻ T lymphocytes (x axis) evaluated in 46 HIV-infected patients; (b) the percentages of CD4⁺CD7⁻CD57⁺ T lymphocytes (x axis) evaluated in 18 HIV-infected patients.

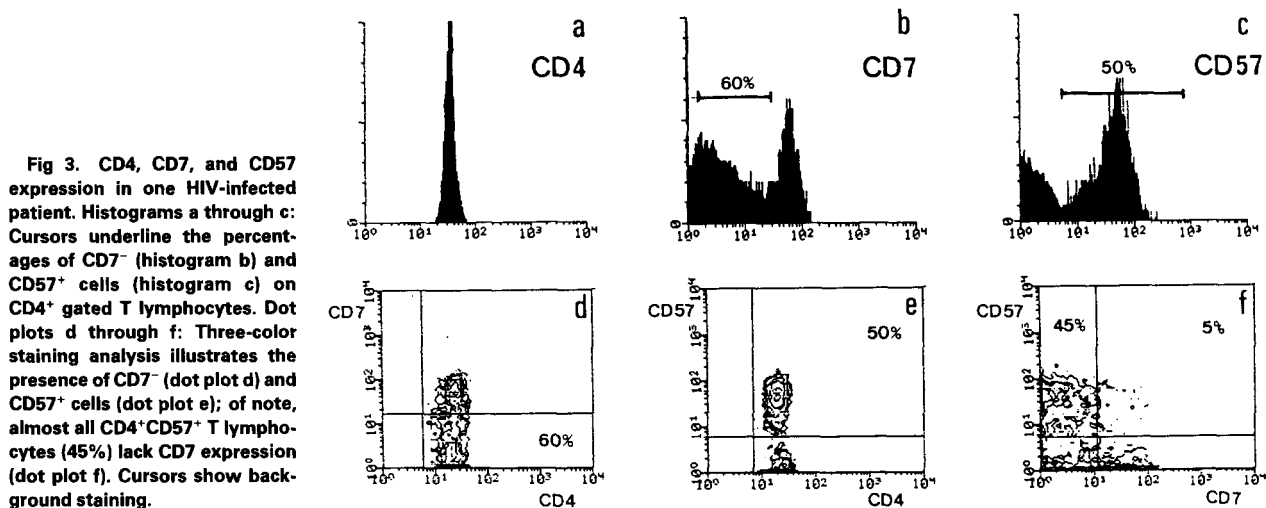


Fig 3. CD4, CD7, and CD57 expression in one HIV-infected patient. Histograms a through c: Cursors underline the percentages of CD7⁻ (histogram b) and CD57⁺ cells (histogram c) on CD4⁺ gated T lymphocytes. Dot plots d through f: Three-color staining analysis illustrates the presence of CD7⁻ (dot plot d) and CD57⁺ cells (dot plot e); of note, almost all CD4⁺CD57⁺ T lymphocytes (45%) lack CD7 expression (dot plot f). Cursors show background staining.

CD45RA.⁵ These data suggest that the lack of CD7 identifies a particular subset of memory helper cells.

The CD7 modulation capacity has been reported. Mainly induced by anti-CD7 MoAbs, this phenomenon is reversible and results in an inhibition of T-cell proliferation.^{24,25} In our technical procedure, CD7 molecule did not modulate and the results of intracytoplasmic staining suggest that there was no CD7 internalization.

The functional role of CD7 remains unclear even if this molecule is supposed to be the T-lymphocyte receptor for IgM.¹⁰ In HIV infection, various antilymphocyte antibodies on CD4⁺ T cells have been reported.²⁶ In such occasions, antilymphocyte IgM could bind to CD7 molecule and induce an epitope masking. To eliminate such a hypothesis, the lack of CD7 expression on CD4⁺TL in HIV-infected patients was controlled by two distinct epitopes of CD7 and no modulation of CD7 antigen was induced by sera from HIV-infected patients.

Previous reports demonstrated that CD7 may function as an accessory activation molecule^{11,27} and that CD7 is a calcium-inducible gene transcribed in immediate stages of activation.²⁸ Heinrich et al²⁹ reported that inhibition of T-cell activation by anti-CD7 MoAb could be helpful in preventing transplant rejection episodes. In our experience, CD4⁺CD7⁻TL do not behave like helper T cells. The absence of proliferative responses at day 2 cannot be explained by cell death because significant responses are observed at day 5. Contrasting with a normal CD2 pathway,

CD4⁺CD7⁻TL weakly respond to anti-CD3 MoAb even if associated with PMA. Interestingly, anti-CD28 MoAb, which delivers a costimulatory signal to T cells,^{30,31} restores the stimulation via CD3 as reported for naive T cells.¹⁷ Altogether, these data suggest that the lack of CD7 cell surface expression observed in normal individuals is not a manifestation of T-cell activation and emphasized the uncommon nature of CD4⁺CD7⁻TL that might correspond to memory cells with an abnormal CD3-TCR stimulation pathway.

Initially described as a marker of neuroectodermal cells,³² the CD57 molecule may be related to neural cell adhesion molecule (NCAM) and myelin associated glycoprotein.¹² Besides NK cells,¹³ CD57 is expressed on a subset of CD8 T lymphocytes, which are supposed to be CD8 suppressor T cells.¹⁴ CD8⁺CD57⁺ cells are expanded in various diseases including HIV disease³³ and after allogeneic bone marrow transplantation.^{34,35} Interestingly, these CD8⁺CD57⁺ cells do express the CD7 antigen.³⁵ More recently, Legendre et al⁴ characterized a subset of CD4⁺CD57⁺ large granular lymphocytes in renal allograft recipients. However, the CD7 expression was not studied. If compared with CD4⁺CD57⁻TL, these cells do not proliferate when stimulated with either lectins or allogeneic cells. Thus, CD57 identifies, in transplant recipients, a subset of CD4 cells that do not behave as previously defined helper cells.

In HIV-infected patients, both the CD4⁺CD7⁻ and CD4⁺CD57⁺ T cells are increased. Our data clearly demon-

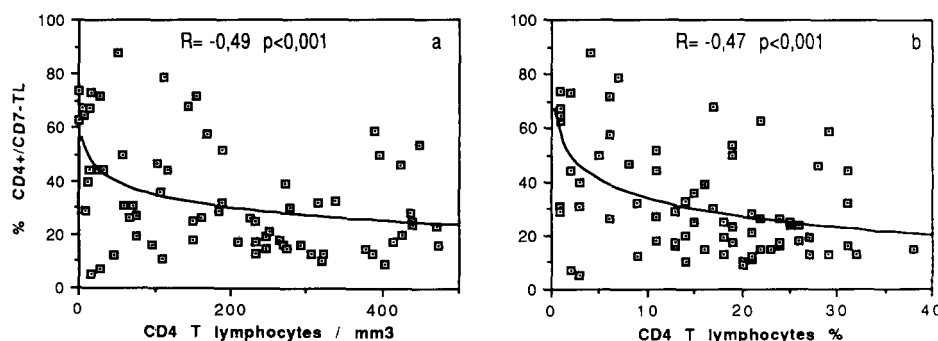


Fig 4. Correlation curves between the percentages of CD4⁺CD7⁻ T lymphocytes (y axis) and (a) the absolute numbers of CD4⁺ T cells/mm³ (x axis); (b) the percentages of CD4⁺ T cells (x axis) evaluated in 78 HIV-infected patients.

strated that the lack of CD7 is strongly correlated with CD57 acquisition on CD4 T cells.

To our knowledge, this is the first description of such a T-cell subset expanded as a function of time in HIV-infected patients. Moreover, the CD4⁺CD7⁻ T cells acquire a prognostic value since correlating with absolute numbers of CD4⁺ lymphocytes. In contrast, because CD57 is detected only on a subset of the CD4⁺CD7⁻TL, its prognostic value remains lower. The low T-cell responsiveness to activation via CD3 observed even at early stages of HIV infection might be related to the expansion of CD4⁺CD7⁻TL.^{18,36} Our results emphasize that, besides quantitative defects, immune deficiency could also be explained by qualitative alterations of CD4⁺ lymphocytes. As previously suggested,¹⁶ the defective function of the CD4⁺ T cells may, in part, account for the imperfect

correlation between cellular immunodeficiency and CD4⁺ T-cell depletion.

The appearance of CD4⁺CD7⁻CD57⁺TL that do not behave as previously defined helper cells raise three important questions: (1) are these cells the result of either a differentiation or activation disorder in T-cell lineage? (2) Are they the result of a selective process related to HIV infection? (3) Are the expression of an unexpected marker (CD57) and the lack of another (CD7) the consequences of an imbalance in cytokines production? These questions are currently under investigation.

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REFERENCES

- Akbar AN, Salmon M, Janosy G: The synergy between naive and memory T cells during activation. *Immunol Today* 12:184, 1991
- Sanders ME, Makgoba MW, June CH, Young HA, Shaw S: Enhanced responsiveness of human memory T cells to CD2 and CD3 receptor-mediated activation. *Eur J Immunol* 19:803, 1989
- Koulova L, Yang SY, Dupont B: Identification of the anti-CD3-unresponsive CD4⁺, CD45RA⁺ peripheral T lymphocytes. *J Immunol* 145:2035, 1990
- Legendre CM, Forbes RDC, Loertscher R, Guttmann RD: CD4⁺/Leu-7⁺ large granular lymphocytes in long-term renal allograft recipients. *Transplantation* 47:964, 1989
- Labastide WB, Rana MT, Barker CR: A new monoclonal antibody (CH-F42) recognizes a CD7⁻ subset of normal T lymphocytes and circulating malignant cells in adult T-cell lymphoma-leukemia and Sézary syndrome. *Blood* 76:1361, 1990
- Haynes BF, Denning SM, Singer KH, Kurtzberg J: Ontogeny of T-cell precursors: A model for the initial stages of human T-cell development. *Immunol Today* 10:87, 1989
- Matutes E, Robinson D, O'Brien M, Haynes BF, Zola H, Catovsky D: Candidate counterparts of Sézary cells and adult T-cell lymphoma-leukemia cells in normal peripheral blood: An ultrastructural study with the immunogold method and monoclonal antibodies. *Leuk Res* 7:787, 1983
- Lindae ML, Abel EA, Hoppe RT, Wood GS: Poikiloderma-tous mycosis fungoides and atrophic large-plaque parapsoriasis exhibit similar abnormalities of T-cell antigen expression. *Arch Dermatol* 124:366, 1988
- Haynes BF, Metzgar RS, Minna JD, Bunn PA: Phenotypic characterization of cutaneous T-cell lymphoma: Use of monoclonal antibodies to compare with other malignant T cells. *N Engl J Med* 304:1319, 1981
- Sandrin MS, Vaughan HA, Lovering KE, Curnow K, Deacon NJ, McKenzie IFC: Characterization of the gene coding for the human lymphocyte surface antigen Huly-m2 (CD7), in McMichael AJ (ed): *Leucocyte Typing III*. Oxford, UK, Oxford University, 1987, p 216
- Chakkalath HR, Jung LKL: The role of CD7 in T cell activation. Federation of American Societies for Experimental Biology, 75th Annual Meeting, Atlanta, GA, April 21-25, 1991. *FASEB J* 5:992, 1991 (abstr)
- Kruse J, Mailhammer R, Wernecke H, Faissner A, Sommer I, Goridis C, Schachner M: Neural cell adhesion molecules and myelin-associated glycoprotein share a common carbohydrate moiety recognized by monoclonal antibodies L2 and HNK-1. *Nature* 311:153, 1984
- Abo T, Balch CM: A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). *J Immunol* 127:1024, 1981
- Lanier LL, Loken ML: Human lymphocyte subpopulations identified by using three-color immunofluorescence and flow cytometry analysis: Correlation of Leu-2, Leu-3, Leu-7, Leu-8 and Leu-11 cell surface antigen expression. *J Immunol* 132:151, 1984
- Velardi A, Mingari MC, Moretta M, Grossi CE: Functional analysis of cloned germinal center CD4⁺ cells with natural killer cell-related features. Divergence from typical T helper cells. *J Immunol* 137:2808, 1986
- Pinching AJ, Nye KE: Defective signal transduction—A common pathway for cellular dysfunction in HIV infection? *Immunol Today* 11:256, 1990
- Miedema F, Tersmette M, van Lier RAW: AIDS pathogenesis: A dynamic interaction between HIV and the immune system. *Immunol Today* 11:293, 1990
- Schellekens PTA, Roos MTL, de Wolf F, Lange JMA, Miedema F: Low T-cell responsiveness to activation via CD3/TCR is a prognostic marker for acquired immunodeficiency syndrome (AIDS) in human immunodeficiency virus-1 (HIV-1)-infected men. *J Clin Immunol* 10:121, 1990
- Ware RE, Scarce RM, Dietz MA, Starmer F, Palker TJ, Haynes BF: Characterization of the surface topography and putative tertiary structure of the human CD7 molecule. *J Immunol* 143:3632, 1989
- Sanders ME, Makgoba MW, Sharrow SO, Stephany D, Springer TA, Young HA, Shaw S: Human memory T lymphocytes express increased levels of three cell adhesion molecules (LFA-3, CD2, and LFA-1) and three other molecules (UCHL1, CDw29, and Pgp-1) and have enhanced IFN- γ production. *J Immunol* 140:1401, 1988
- Sagawa K, Hagiwara S, Yasuda K, Yokoyama MM: Expression profiles of T-cell associated antigens on adult T-cell leukemia cells: Results of a workshop study, in Reinherz EL, Haynes BF, Nadler LM, Bernstein ID (eds): *Leucocyte Typing II*, vol 1. Boston, MA, Springer-Verlag, 1986, p 361
- Lazarovits AI, White MJ, Karsh J: CD7 negative T cells in rheumatoid arthritis. Federation of American Societies for Experimental Biology, 75th Annual Meeting, Atlanta, GA, April 21-25, 1991. *FASEB J* 5:963, 1991 (abstr)
- Jung LKL, Fu SM, Hara T, Kapoor N, Good RA: Defective expression of T-cell associated glycoprotein in severe combined immunodeficiency. *J Clin Invest* 77:940, 1986

24. Lazarovits AI, Colvin RB, Camerini D, Karsh J, Kurnick JT: Modulation of CD7 is associated with T-lymphocyte function, in McMichael AJ (ed): *Leukocyte Typing III*. Oxford, UK, Oxford University, 1987, p 219
25. Emara M, Azari K, Sanfilippo F: Inhibition of T-cell proliferation by modulation of CD7 or IgM-Fc receptors. Federation of American Societies for Experimental Biology, 75th Annual Meeting, Atlanta, GA, April 21-25, 1991. *FASEB J* 5:994, 1991 (abstr)
26. Daniel V, Schimpf K, Opelz G: Lymphocytes autoantibodies and alloantibodies in HIV-positive haemophilia patients. *Clin Exp Immunol* 75:178, 1989
27. Carrera AC, Rincon M, Sanchez-Madrid F, Lopez-Botet M, de Landazuri MO: Triggering of co-mitogenic signals in T cell proliferation by anti-LFA-1 (CD18, CD11a), LFA-3, and CD7 monoclonal antibodies. *J Immunol* 141:1919, 1988
28. Ware RE, Haynes BF: CD7 is a calcium-inducible gene transcribed in the immediate stages of T cell activation. Federation of American Societies for Experimental Biology, 75th Annual Meeting, Atlanta, GA, April 21-25, 1991. *FASEB J* 5:991, 1991 (abstr)
29. Heinrich G, Gram H, Kocher HP, Schreier MH, Ryffel B, Akbar A, Amlot PL, Janosy G: Characterization of a human T cell-specific chimeric antibody (CD7) with human constant and mouse variable regions. *J Immunol* 143:3589, 1989
30. Vanderberghe P, Fletcher MC, Turka LA, Thompson CB, June CH: Anti-CD28 mAb 9.3 induces tyrosine phosphorylation in human T cells. Federation of American Societies for Experimental Biology, 75th Annual Meeting, Atlanta, GA, April 21-25, 1991. *FASEB J* 5:991, 1991 (abstr)
31. Bjorndahl JM, Sung SSJ, Hansen JA, Fu SM: Human T cell activation: Differential response to anti-CD28 as compared to anti-CD3 monoclonal antibodies. *Eur J Immunol* 19:881, 1989
32. Vincent M, Thiery JP: A cell surface marker for neural crest and placodal cells: Further evolution in peripheral and central nervous system. *Dev Biol* 103:468, 1984
33. Sadat-Sowti B, Debre P, Idziorek T, Guillon JM, Hadida F, Okzenhendler E, Katlama C, Mayaud C, Autran B: A lectin-binding soluble factor released by CD8⁺CD57⁺ lymphocytes from AIDS patients inhibits T cell cytotoxicity. *Eur J Immunol* 21:737, 1991
34. Leroy E, Calvo CF, Divine M, Gourdin MF, Baujean F, Ben Aribia MH, Mishal Z, Vernant JP, Farcet JP, Sennik A: Persistence of T8⁺/HNK-1⁺ suppressor lymphocytes in the blood of long-term surviving patients after allogeneic bone marrow transplantation. *J Immunol* 137:2180, 1986
35. Autran B, Leblond V, Sadat-Sowti B, Lefranc E, Got P, Sutton L, Binet JL, Debre P: A soluble factor released by CD8⁺CD57⁺ lymphocytes from bone marrow transplanted patients inhibits cell mediated cytolysis. *Blood* 77:2237, 1991
36. Gruters RA, Terpstra FG, De Jong R, Van Noesel CJM, Van Lier RAW, Miedema F: Selective loss of T cell functions in different stages of HIV infection. *Eur J Immunol* 20:1039, 1990