## Polyclonal T-cell expansions in a HIV+ patient with atypical cutaneous lymphoproliferative disorder, large granular lymphocyte proliferation and SENV infection

**Lesional and peripheral lymphocytes of a HIV+ patient who, at a one-year interval, exhibited an atypical cutaneous lymphoproliferative disorder and an expansion of large granular lymphocytes, showed predominant expression of polyclonal or slightly oligoclonal TCRBV2 chain. Circulating SENV-A and SENV-H, two strains of a recently identified blood-borne DNA virus, were detected, respectively, at the time the skin lesion manifested and LGL proliferation.**

The absence of clonal T-cell receptor (TCR) gene rearrangements in CD8+ lymphocytes found in the skin lesions of HIV+ patients with atypical cutaneous lymphoproliferative disorder (ACLD) suggested that HIV-associated ACLD is a reactive rather than a malignant process.[1,2] Similarly, in HIV+ patients, the remarkable restriction of TCR repertoire of CD8+CD57+ cells with large granular lymphocyte (LGL) morphology[3,4] indicated that these lymphocytes may represent a cytolytic population that continuously arises during viral infections.[5]

The mechanisms that induce cutaneous accumulation of CD8 lymphocytes and peripheral expansion of CD8+CD57+ LGL in HIV+ patients remain unexplained. The identification of a HIV+ patient who, at a one-year interval, showed both ACLD and LGL proliferation allowed us to investigate the possibility of a common causative antigen responsible for the induction of T-cell abnormalities. This bisexual patient was always in good general condition but, in June 1996, he was referred to Brescia hospital because of multiple, reddish-pink cutaneous nodular lesions, located on the sacral, gluteal regions and thighs that, after 4 months, regressed without treatment. The dominant histologic features of the nodular lesions consisted of a dense cell infiltrate composed of atypical small lymphocytes, eosinophils and rare neutrophils. The lymphocytes showed epidermotropism and were CD3+ (Figure 1a), most of which were also CD8+, CD5+, CD43+ and CD45RO+ and about 10% expressed CD57 antigen.

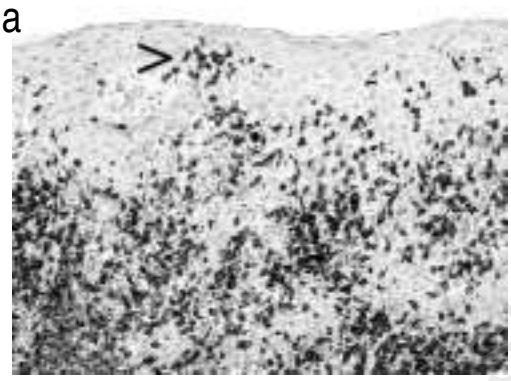


**Figure 1. a) Skin biopsy stained with anti-CD3, showing a dense lymphoid infiltrate in the superficial dermis with focal epidermotropism (arrow). b) left: TCRBV repertoire of lesion-infiltrating lymphocytes. The analysis was performed by a degenerate PCR, followed by a colorimetric assay. Middle: heteroduplex analysis of the indicated TCRBV chains. TCRBV8 PCR products, obtained from amplification of *J77* and C1-632 cells, were used as monoclonal and polyclonal controls and are shown at the left. Right: Sequences of the TCRBV2 segment. c) Left: TCRBV repertoire of CD4+, CD8+ CD57+ and CD8+ CD57− subpopulations. The analysis was performed by a degenerate PCR, followed by a colorimetric assay. Middle: heteroduplex analysis of the indicated TCRBV chains obtained from different populations: (+) = CD4+ lymphocytes, (++) = CD8+CD57+ lymphocytes and (–) = CD4−CD57− lymphocytes. Right: Sequences of the TCRBV2 segment.**

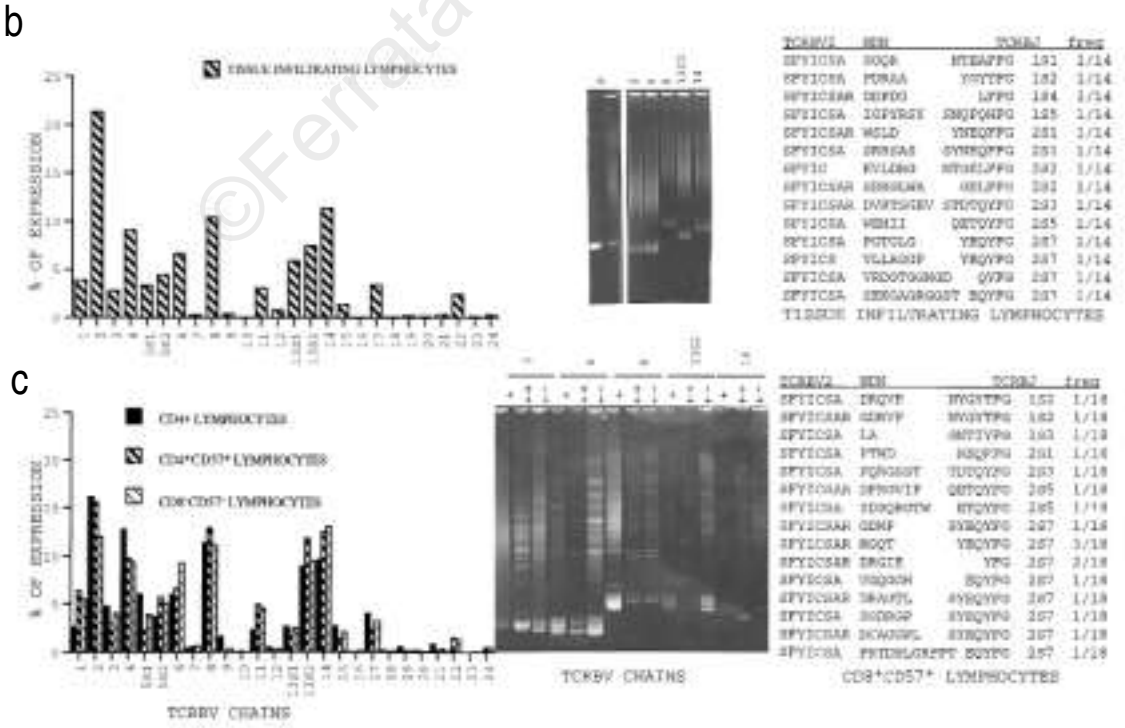

**Table 1. Laboratory results.**

| Date | CD3 | CD4 | CD8 | CD8+ CD57+ % ($mm^3$) | LGL | HIV-RNA (copies/mL) | SENV-DNA strain A | D | H |
|---|---|---|---|---|---|---|---|---|---|
| July 1996 | 90 (1602) | 14 (249) | 80 (1424) | nd | 1 (18) | 33,000 | + | – | – |
| July 1997 | 81 (1514) | 12 (224) | 62 (1139) | 35 (654) | 41 (766) | 33,700 | – | – | + |
| March 1998 | 90 (1593) | 15 (265) | 67 (1186) | 28 (496) | 14 (248) | 8,700 | – | – | – |
| July 1998 | 84 (1512) | 15 (270) | 62 (1116) | 13 (234) | 6 (108) | 2,300 | – | – | – |
| December 1999 | 86 (1895) | 17 (375) | 62 (1388) | 22 (484) | 18 (396) | 5,300 | – | – | + |
| Healthy controls | 77±7 | 49±89 | 27±7 | 10±4 | 12±5 | – | – | – | – |

At the time of ACLD no atypical CD8 lymphocytes were observed in the blood smear, while one year later, 41% of $CD8^+$ lymphocytes showed LGL morphology and 35% were $CD57^+$. Forty-four percent of these lymphocytes were also $CD28^-$, 29% were $DR^+$ and 8% displayed the natural killer (NK)-associated CD16 antigen. $CD57^+$ LGL expression progressively decreased in the following months but, in December 1999, they increased again above the normal range (Table 1). Therefore, while the course of cutaneous disease and LGL proliferation in HIV patients is persistent or progressive,[1,3] our patient's skin lesions regressed without treatment and LGL expansion was not stable over time. The transitory accumulation of $CD8^+$ cells in the skin and the increase of LGL were apparently not related to modification of HIV viremia or associated with acute viral infections that often afflict $HIV^+$ patients (Table 1) and that may induce abnormal T-cell proliferation.[3,6,7] However, SENV-A, widely present in $HIV^+$ patients, and SENV-H, that it has been indicated as putative agent of transfusion-associated hepatitis,[8,9] were respectively identified just at the time of ACLD and LGL proliferations. Since the SENV family is composed of a number of viruses clearly distinguishable by their heterogeneity in terms of both genetic structure and biological properties, it is also possible that SENV-A and SEN-H show different tropism.

As increases in both ACLD and LGL could be the result of immunologic reactions to viral antigens, the fine characterization of the TCR repertoire is an appropriate approach for determining the dynamics of the immune response to foreign antigens. This analysis, performed as previously reported,[4] demonstrated that the TCRBV2 segment was dominantly expressed by the skin-infiltrating lymphocytes (Figure 1b, left). Heteroduplex analysis and sequencing showed that the TCRBV2-specific product migrated in a polyacrylamide gel as smears and that all TCRBV2 cDNA clones were different (Figure 1b, middle and right), indicating the polyclonality of $TCRBV2^+$ lymphocytes. The TCRBV2 segment was also largely represented in the T-cells subsets prepared at the moment of LGL expansion (Figure 1c, left). $CD8^+CD57^+$ cells did not, however, show the clear-cut dominance of a single or few TCRBV chains that is found in other LGL proliferations.[4,10] Furthermore, in contrast to other patients with increased numbers of LGL, heteroduplex analysis demonstrated that the dominant TCRBV2 population prepared from the $CD8^+CD57^+$ subset migrated in the heteroduplex gel as faint homoduplex bands, often superimposed on smears, suggesting the presence of only minor clonal populations in the context of a background of polyclonal T-cells (Figure 1c, middle). These data were confirmed by sequencing because two minor clones were found within different $CD8^+CD57^+$ sequences (Figure 1c, right).

Although the pathologic relevance of SENV still needs to be assessed, the transition from a negative to a positive SENV DNA state may have induced, in our patient, a polyclonal or slightly oligoclonal T-cell proliferation resulting in skin and peripheral blood abnormalities simulating lymphoproliferative diseases. Therefore, viruses with unknown biological or pathologic significance in healthy subjects may be capable of modulating T-cell responses in immunodeficient patients, in predisposed hosts or under particular conditions.

*Silvia Pirovano,* Liana Signorini,° Fabio Facchetti,# Amerigo Santoro,# Alberto Albertini,*@ Luisa Imberti@*

**Institute of Chemistry, #Pathology, and °Infectious Diseases, School of Medicine, University of Brescia; @Terzo Servizio Analisi, A.O. Spedali Civili, Brescia, Italy*

*Key words: cutaneous lymphoproliferation, human immunodeficiency virus, large granular lymphocytes, SEN virus.*

*Funding: Supported by Istituto Superiore della Sanità, AIDS Project 1999, and by DiaSorin, Saluggia, Italy.*

*Correspondence: Luisa Imberti, M.D., Laboratorio di Biotecnologie, Terzo Laboratorio Analisi, A.O. Spedali Civili, p.le Spedali Civili, 1, 25123 Brescia, Italy. Phone: international +39.030.3996427. Fax: international +39.030.307251. E-mail: limberti@spidernet.it*

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