Benign monoclonal expansion of CD8+ lymphocytes in HIV infection

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Abstract

Background—A transient expansion of the CD8+ T cell pool normally occurs in the early phase of HIV infection. Persistent expansion of this pool is observed in two related settings: diffuse infiltrative lymphocytosis syndrome (DILS) and HIV associated CD8+ lymphocytosis syndrome.

Aim—To investigate a group of HIV infected patients with CD8+ lymphocytosis syndrome with particular emphasis on whether monoclonality was present.

Methods—A group of 18 patients with HIV-1 infection and persistent circulating CD8+ lymphocytosis was compared with 21 HIV positive controls. Serum samples were tested for antinuclear antibodies, antibodies to extractable nuclear antigens, immunoglobulin levels, paraproteins, human T lymphotropic virus type 1 (HTLV-1), Epstein-Barr virus, and cytomegalovirus serology. Lymphocyte phenotyping and HLA-DR typing was performed, and T cell receptor (TCR) gene rearrangement studies used to identify monoclonal populations of T cells. CD4+ and CD8+ subsets of peripheral blood lymphocytes were purified to determine whether CD8+ populations inhibited HIV replication in autologous CD4+ cells.

Results—A subgroup of patients with HIV-1 infection was found to have expanded populations of CD8+ T cell large granular lymphocytes persisting for 6 to 30 months. The consensus immunophenotype was CD4− CD8+ DRαβ CD11a+ CD11c+ CD16− CD56− CD57+, consistent with typical T cell large granular lymphocytes expressing cellular activation markers. Despite the finding of monoclonal TCR gene usage in five of 18 patients, there is evidence that the CD8+ expansions are reactive populations capable of mediating non-cytotoxic inhibition of HIV replication.

Conclusions—A subgroup of HIV positive patients has CD8+ lymphocytosis, but despite the frequent occurrence of monoclonal TCR gene usage there is evidence that this represents an immune response to viral infection rather than a malignant disorder.

Keywords: HIV infection; CD8+ lymphocytosis; clonality

Infection with HIV causes a progressive depletion of CD4+ T cells and subsequent immune deficiency, developing at variable rates in individual patients. The CD8+ T cell population is expanded in early HIV infection as part of the host immune response to the virus. This response is generally not sustained and is followed by a subsequent decline in CD8+ T cell numbers.

B cell lymphomas are found commonly in patients with HIV infection, while T cell malignancy is extremely uncommon. However, there are two clinical settings in which expansion of the CD8+ T cell population has been identified in association with HIV infection: the first is HIV associated CD8+ lymphocytosis syndrome and the second is diffuse infiltrative lymphocytosis syndrome (DILS), a syndrome which may represent a subgroup of the former. DILS is characterised by the sequestration of CD8+ T cells in the salivary glands, lungs, kidneys, and other visceral sites and clinically resembles Sjögren’s syndrome, although characteristically autoantibodies such as antinuclear antibodies (ANA), anti-Ro, and anti-La are absent. DILS patients have an increased frequency of certain major histocompatibility complex (MHC) class II alleles: HLA-DRB1*1301 among blacks and Europids and HLA-DRB1*1102 among blacks only. This finding is consistent with the hypothesis that DILS is a florid immune response to HIV infection, restricted by the ability of specific MHC alleles to present HIV peptides to the T cell receptor (TCR).

Examination of the peripheral blood in patients with DILS has shown CD8+ T cell populations consisting primarily of a memory/effector phenotype, characterised by increased CD57 expression along with the cell adhesion molecules CD11a/CD18 and CD29. In T cells infiltrating the salivary glands of HIV positive individuals with DILS, TCR β usage is restricted. There is preferential usage of certain non-germline amino acids at position...
immunoglobulin levels and the presence of extractable nuclear antigens (ENA) by indirect immunofluorescence and antibodies. All patients underwent testing for ANA by ELISA and were associated with an improved long-term prognosis, defined by the presence of at least three months of at least $1.5 \times 10^9/l$ CD8+ T cells. We have investigated a group of HIV infected patients with persistent circulating CD8+ lymphocytosis. Our aim was to determine the immunophenotype of these expanded populations and to explore the relation of this syndrome with the better described DILS clinical phenotype. We were particularly interested in the clonality of these expanded T cell populations and whether antiviral activity could be demonstrated.

**Methods**

**PATIENTS**

Patients with HIV-1 infection and persistent circulating CD8+ lymphocytosis were compared with a group of 21 HIV positive controls matched by age, sex, route of transmission, and ethnic origin in July 1998. The patients attended the Grahame Hayton Unit at the Royal London Hospital with HIV infection diagnosed by enzyme linked immunosorbent assay (ELISA) and western blot analysis. Case records of all 432 patients were reviewed and of these, 20 were found to have CD8+ lymphocytosis, defined as a CD8+ count of greater than $1.5 \times 10^9/litre$ persisting for more than three months. One patient was excluded from the study owing to active hepatitis B infection, which is a well recognised cause of pre-existing CD8+ lymphocytosis. Other exclusion criteria included known HIV seroconversion within the previous three months.

Informed consent was obtained, according to the procedure laid down by the institution's ethics committee, from 18 of the patients who were suitable for study. Those with CD8+ lymphocytosis were evaluated for the presence of sicca symptoms including a Schirmer's test, and a physical examination was carried out to identify the presence of parotid gland enlargement. Blood samples were collected on enrolment and where possible serological data were obtained from medical records, along with details of antiretroviral treatment.

**SEROLOGICAL STUDIES**

All patients underwent testing for ANA by indirect immunofluorescence and antibodies against extractable nuclear antigens (ENA) by gel diffusion. Serum samples were tested for immunoglobulin levels and the presence of paraproteins. Twelve of the 18 patients with CD8+ lymphocytosis consented to HTLV-1 testing, which was carried out using a passive particle agglutination test to detect antibodies to HTLV-1. Epstein-Barr virus (EBV) VCA IgG was performed on all patients from stored serum samples. Serological data obtained from medical records included hepatitis B and C serology and testing for the presence of cytomegalovirus (CMV) IgM antibody.

**LYMPHOCYTE PHENOTYPIC ANALYSIS**

Peripheral blood lymphocyte subsets were analysed in all patients by flow cytometry on lysed whole blood.

**HLA TYPING**

HLA typing for DRB was performed on 17 patients with CD8+ lymphocytosis and the matched control group of 21 HIV positive individuals using sequence specific primers.

**TCR GENE REARRANGEMENT STUDIES**

Peripheral blood samples were collected in EDTA on all patients. Following DNA extraction, single stranded conformation polymorphism polymerase chain reaction was used to analyse TCR γ gene rearrangements. This technique is required because of the limited combinatorial diversity of the TCR γ locus, as it detects variations in both sequence and size of amplified DNA.

**p24 INHIBITION STUDIES**

Inhibition studies of p24, an HIV core protein, were performed to determine whether CD8+ populations could inhibit HIV replication in autologous CD4+ cells. These studies were performed on two control group patients and five patients with high circulating levels of CD8+ T cells. Peripheral blood lymphocytes were density separated and monocytes removed by adhesion. B and CD8+ T cells were removed using magnetic beads. The remaining CD4+ T cells were resuspended at $10^6/ml$ in RPMI, 10% autologous serum, and antibiotics. CD8+ T cells were then added back to CD4+ T cells at proportions of 0%, 6.25%, 25%, or 50%. The CD4+/CD8+ mixtures were stimulated with PHA 5 µg/ml for eight days. Supernatant was harvested and the p24 concentration determined by ELISA (Coulter).

**STATISTICAL ANALYSIS**

Statistical analysis included descriptive statistics of the study population, and the Mann-Whitney U test was used where continuous data were not normally distributed. These tests of significance were two tailed. Categorical data were compared using the $\chi^2$ test. The level of significance was set at $p < 0.05$.

**Results**

**PATIENTS**

The median age of the control group was 32 years, and that of the CD8+ lymphocytosis group 40.5 years (table 1). The observed duration of continuous CD8+ lymphocytosis was six to 30 months. In the CD8+ lymphocytosis group, two of the 18 patients complained of...
sicca symptoms requiring artificial tears, though Schirmer’s test was negative in both. There was no clinical evidence of parotid gland enlargement, splenomegaly, or neutropenia in either patient group. From the CD8+ lymphocytosis group, 15 of the 18 patients (83.3%) were taking combination antiretroviral treatment, as were 17 of the 21 patients from the control group (81.0%). Patients with CD8+ lymphocytosis had been infected with HIV-1 for significantly longer than controls at time of entry to the study. The median HIV-1 RNA viral load was slightly lower in the CD8+ lymphocytosis group (p = 0.4, NS).

LYMPHOCYTE PHENOTYPIC ANALYSIS
The consensus immunophenotype of the CD8+ T cells was CD3+ CD4− CD8+ Dr^high CD11a+ CD11c+ CD16− CD28± CD56− CD57+ (table 1). This is the expected immunophenotype of T cell large granular lymphocytes as seen on peripheral blood smears (fig 1), and the enhanced DR expression is suggestive of cellular activation.

TCR GENE REARRANGEMENT STUDIES
Clonal analysis results are shown in table 1. Five patients from the CD8+ lymphocytosis group had monoclonal T cell populations, of whom two also had paraproteins. All five of these patients were taking antiretroviral treatment at this time.

SEROLOGICAL AND HLA STUDIES
None of the patients tested had antibodies against HTLV-1 or hepatitis C virus, and all patients tested were positive for CMV IgG. ANA was weakly positive at 1:80 in three of the 18 patients with CD8+ lymphocytosis and two of the 21 controls, all corresponding to a homogeneous pattern. Anti-Ro and anti-La antibodies were not detected in either patient group. The median total IgG level was slightly higher in the CD8+ lymphocytosis group (table 1); however, this difference did not reach statistical significance.

Table 1 Demographic data, dual immunophenotyping studies, presence of paraproteins, and TCR gene rearrangement results in control and CD8+ lymphocytosis groups

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=21)</th>
<th>CD8+ lymphocytosis (n=18)</th>
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</thead>
<tbody>
<tr>
<td>Age range (years)</td>
<td>20 to 55</td>
<td>30 to 58</td>
</tr>
<tr>
<td>Race</td>
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<td></td>
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<tr>
<td>Europid</td>
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<td>14</td>
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<tr>
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<td>4</td>
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<tr>
<td>Asian</td>
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<td>0</td>
</tr>
<tr>
<td>Mode of transmission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male to male</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Heterosexual</td>
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<td>4</td>
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<tr>
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</tr>
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<td>5</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Median time since diagnosis (years)</td>
<td>4.5</td>
<td>8.0*</td>
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<tr>
<td>No of patients taking antiretroviral treatment</td>
<td>17 (81.0%)</td>
<td>15 (83.3%)</td>
</tr>
<tr>
<td>Median HIV-1 RNA viral load (copies/ml)</td>
<td>3511</td>
<td>1172†</td>
</tr>
<tr>
<td>Paraproteins</td>
<td>0</td>
<td>2</td>
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<tr>
<td>(Patient No 1, IgG and IgM)</td>
<td>(Patient No 2, IgG)</td>
<td></td>
</tr>
<tr>
<td>Median IgG level (g/litre)</td>
<td>15.8</td>
<td>17.25‡</td>
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<tr>
<td>Lymphocyte phenotype double positive (mean % of T cells)</td>
<td></td>
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<tr>
<td>CD8+ + DR+</td>
<td>35.7</td>
<td>63.2</td>
</tr>
<tr>
<td>CD8+ + CD11a+</td>
<td>63.7</td>
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<tr>
<td>CD8+ + CD11c+</td>
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<td>18.4</td>
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<td>CD8+ + CD57+</td>
<td>26.5</td>
<td>28.2</td>
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<tr>
<td>EBV VCA IgG positive (No of patients)</td>
<td>14 (66.7%)</td>
<td>13 (72.2%)</td>
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<td>TCR gene rearrangement‡</td>
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*p=0.0007, Mann-Whitney U test.
†p=0.4, Mann-Whitney U test.
‡p=0.23, χ² (normal range IgG < 16 g/l).
*χ²=0.008, χ².

Discussion
This group of patients with persistent CD8+ lymphocytosis is clearly distinct from those patients with DILS who have been reported previously. The former group did not show a preferential association with certain HLA alleles or parotid gland enlargement, and with the exception of two patients, sicca symptoms were not a prominent feature. There is evidence that CD8+ lymphocytosis occurring in association within both groups. ANA was weakly positive at 1:80 in three of the 18 patients with CD8+ lymphocytosis two of the 21 controls, all corresponding to a homogeneous pattern. Anti-Ro and anti-La antibodies were not detected in either patient group. The median total IgG level was slightly higher in the CD8+ lymphocytosis group (table 1); however, this difference did not reach statistical significance.

The HLA alleles linked to DILS were seen more often in controls (DRB1*11+, 8/21; DRB1*13+, 6/21) than in patients with CD8+ lymphocytosis (DRB1*11+, 3/17; DRB1*13+, 1/17).

Figure 1 Peripheral blood film showing T cell large granular lymphocytes in a patient with CD8+ lymphocytosis (×270).
with DILS represents an antigenically driven immune response which is associated with a slow progression of HIV disease.\(^1\) \(^9\) CD8\(^+\) lymphocytosis may be a part of the spectrum of this syndrome, and its presence should alert the clinician to the possibility of the presence of visceral infiltration by CD8 lymphocytes.

Although there are previous reports of non-DILS CD8\(^+\) T cell expansions occurring in the setting of HIV infection, our series adds considerably to the reported number of cases of HIV associated CD8\(^+\) lymphocytosis where a monoclonal TCR gene rearrangement has been detected. In an analysis of 2363 HIV positive individuals, CD8\(^+\) lymphocytosis was detected in only eight cases, and all of these were found to have polyclonal TCR \(\beta\) chain rearrangements.\(^15\) Furthermore, all eight patients had undergone a splenectomy following abdominal trauma and it is well known that lymphocytosis is a frequent post-splenectomy finding. In a more recent case report,\(^16\) a monoclonal expansion of T cell large granular lymphocytes, as determined by TCR gene rearrangement studies in a splenectomised HIV positive patient, was interpreted as indicating a T cell leukaemia, although no clinical evidence of malignancy was described other than lymphocytosis. Similarly, none of the patients that we report has shown clinical evidence of malignancy on follow up to 12 months, and in some instances the CD8\(^+\) counts fell after a period of antiretroviral treatment (data not shown). Five patients from the CD8\(^+\) lymphocytosis group were shown to have monoclonal T cell populations; all the patients in this subgroup were on antiretroviral treatment at the time this study was carried out. This subgroup included two female black African patients with paraproteins. These women underwent a bone marrow examination which showed increased lymphocytes but no evidence of malignancy, and an abdominal ultrasound scan which did not show hepatosplenomegaly or lymphadenopathy. Urinary free light chains were not detected in either of these patients.

Similar small monoclonal CD8\(^+\) T cell populations have also been seen in normal elderly individuals and in infants with HIV infection.\(^10\) \(^17\) and a larger transient proliferation of CD8\(^+\) T cells with monoclonal gene rearrangement of the TCR \(\beta\) locus has been described in acute viral hepatitis.\(^16\) Oligoclonal patterns of TCR gene rearrangements have also been reported in patients with acute EBV induced infectious mononucleosis.\(^19\) We conclude that, despite the presence of monoclonal TCR gene rearrangements, there is no additional evidence to suggest the presence of a malignant process in this group of patients with monoclonal TCR gene rearrangements. Rather, our findings support the important general point that clonality itself is not necessarily indicative of malignancy.

In our group of patients B cell activation is evident. Patients with the highest CD8\(^+\) T cell numbers were more likely to have monoclonal T cell populations and tended to have the highest immunoglobulin levels, along with detectable paraproteins in two cases, suggesting that common factors drive both T cell and B cell expansions. There was no correlation between immunoglobulin levels and previous exposure to EBV or CMV, and it can be seen that although median duration of HIV infection was significantly longer in those with CD8\(^+\) lymphocytosis than in controls, the groups were otherwise similar. Therefore, although oligoclonal and clonal T cell expansions quite commonly occur during immune responses, we were unable to correlate CD8\(^+\) T cell expansions in HIV infected patients with any other common viral infection.

In this group of patients the expanded populations have the phenotype CD8\(^+\) DR\(^+\) LFA1\(^+\) CD28\(^+,\) which is certainly not typical of T cell malignancies. The CD8\(^+\) T cells in this group appear to be reactive, as suggested by their high DR expression. Furthermore there is evidence that the CD8\(^+\) T cells in our patients inhibit HIV replication. The expanded CD8\(^+\) population may therefore represent an attempt to restore reactivity to HIV. CD8\(^+\) T cell mediated suppression of HIV replication measured in the same way in early perinatal HIV infection has potent effects on viral load and survival.\(^20\) There is circumstantial evidence to suggest that the expanded
CD8+ lymphocytes in HIV infection

CD8+ T cell numbers in HIV associated CD8+ lymphocytosis syndrome are involved in an immune response directed at an antigen derived from HIV itself. It has been reported that this process may be partly mediated by a soluble lymphokine, acting in a bystander fashion.

Although it was previously thought that most of the cells contributing to a virally induced lymphocytosis are bystanders, studies in murine models have shown that large numbers of such cells are specific for viral peptides. Since precursor cells for specific peptides are rare, a brisk 10-fold expansion of responding cells is required to explain these findings. New MHC/tetramer technology suggests that a high frequency of HIV responding T cells is required to explain these findings. Newiset, UK.

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