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T cell activation and disease severity in HIV infection

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SUMMARY

In vitro studies have indicated that T lymphocyte activation may be of importance in the pathogenesis of HIV infection. In order to define the role of immune activation *in vivo*, we assessed the expression of the T cell activation markers HLA-DR and CD25 by flow cytometry in peripheral blood in relation to disease severity and the surrogate markers CD4 and β_2-microglobulin in 157 patients with HIV infection and 53 healthy seronegative blood donors. Percentage levels of $CD3^+HLA\text{-}DR^+$ T lymphocytes were significantly higher ($P<0.0001$) and percentage levels of $CD3^+CD25^+$ T lymphocytes significantly lower ($P<0.0001$) in all HIV^+ patients compared with controls. A significant correlation was observed between increasing percentage levels of $CD3^+HLA\text{-}DR^+$ T lymphocytes and both declining CD4 counts ($r=0.52$; $P<0.001$) and increasing β_2-microglobulin levels ($r=0.56$; $P<0.001$). Percentage levels of $CD4^+HLA\text{-}DR^+$ and $CD4^+CD25^+$ lymphocytes were significantly higher in all HIV^+ patients compared with controls ($P<0.001$). Levels of activated ($HLA\text{-}DR^+$ and $CD25^+$) $CD4^+$ lymphocytes showed a significant step-wise linear increase with increasing disease severity ($P<0.001$). High levels of $CD3^+HLA\text{-}DR^+$ T lymphocytes were found in a greater proportion (81·8%) of asymptomatic HIV^+ patients (Centres for Disease Control (CDC) group II) than low CD4 counts (51·5%) ($P<0.001$). Compared with controls, HIV^+ patients had higher percentage levels of $CD8^+HLA\text{-}DR^+$ lymphocytes ($P<0.001$), but similar levels of $CD8^+CD25^+$ lymphocytes. These results indicate that T cell activation is not only a consistent but also an early feature in HIV infection. Monitoring levels of activated T cells and their subsets is of value in assessing progression of HIV-related disease.

Keywords HIV immune activation disease progression

INTRODUCTION

In vitro evidence suggests that activation of the cellular immune system plays a central role in promoting the progression of HIV infection. $CD4^+$ lymphocytes are more susceptible to infection by HIV when activated than when in a resting state, and both HIV entry and HIV envelope-dependent cell–cell fusion appear to require T cell activation [1,2]. The activation of T lymphocytes chronically infected with HIV *in vitro* in turn, triggers the transition from latency to active viral replication [3]. If these activation-dependent infection-promoting events occur not only *in vitro* but also *in vivo*, the study of T cell activation may provide both pathogenic insights and markers of disease severity in HIV infection.

T cell activation can be assessed by the study of surface molecules usually absent or expressed at a very low level on quiescent T cells, such as HLA-DR and CD25 (IL-2 receptor, IL-2R). HLA-DR is an MHC class II antigen and is expressed on T cells after antigenic or mitogenic stimulation, reaching a peak after approximately 7 days. The receptor for the T cell growth and activation factor IL-2, on the other hand, is induced earlier, with peak expression occurring at 48–72 h.

To ascertain the degree of T cell activation and its relationship to disease severity in HIV infection, we measured the expression of these markers on the total T lymphocyte population and on the helper/inducer ($CD4^+$) and suppressor cytotoxic ($CD8^+$) T cell subsets in HIV^+ patients with disease of varying severity, using two-colour flow cytometry. To examine further the relationship between T cell activation and disease severity, activation markers were compared with accepted indices of disease progression, namely CD4 count and β_2-microglobulin, and with neopterin, a marker of both disease progression and cellular immune activation.

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PATIENTS AND METHODS

Subjects

One hundred and fifty-seven patients with HIV infection (150 males, 7 females; median age 29 years, range 21–42 years) in whom the diagnosis was made on the basis of positive testing for

the presence of anti-p24 antibody (Wellcome Diagnostics, Dartford, UK) and p24 antigen (Abbott Diagnostics, Maidenhead, UK) were studied. One hundred and twenty-nine patients were positive for anti-p24 antibody alone, 27 patients were positive for p24 antigen alone, and one patient was positive for both antigen and antibody. When divided according to the Centres for Disease Control (CDC; Atlanta, GA) classification, 66 patients fell in the asymptomatic group (CDC group II), 45 in the persistent generalized lymphadenopathy group (PGL; CDC group III), 10 had ARC (CDC group IVA), and 36 had AIDS (CDC group IVB–E). Fifty-three healthy HIV^- blood donors recruited from the South-East Thames Regional Blood Transfusion Service were used as normal control subjects (51 males, 2 females; median age 34 years, range 22–44 years).

In all 157 HIV^+ patients and the 53 seronegative controls T cell activation was assessed on $CD3^+$ T lymphocytes, using the activation markers HLA-DR and CD25.

In 49 HIV^+ individuals (48 males; median age 31 years, range 23–38 years) and 28 healthy seronegative blood donors (28 males; median age 32 years, range 22–38 years) activation of $CD4^+$ and $CD8^+$ T cell subsets was also studied (20 in CDC group II, 15 in CDC III and 14 in CDC IVA–E).

Lymphocyte phenotype analysis

Two-colour immunophenotyping of peripheral blood lymphocytes (PBL) was performed by flow cytometry using a lysed whole blood technique and a FACScan (Becton Dickinson, Mountain View, CA). MoAbs conjugated with either FITC or PE were used. Briefly, 100 μl of whole blood taken into 10 mmol/*l* of ethylenediamine tetra-acetic acid (EDTA) were incubated with saturating amounts of the MoAbs at room temperature in the dark for 15 min. Two millilitres of lysing solution (Becton Dickinson) were then added and incubated for a further 10 min. Cells were then washed twice in PBS pH 7·2, and finally resuspended in 500 μl of 0·5% paraformaldehyde in FACSflow sheath fluid (Becton Dickinson). The following combinations of MoAbs were used: PE-conjugated anti-HLA-DR or anti-CD25 in combination with FITC-conjugated anti-CD3, CD4 or CD8 (all Becton Dickinson).

A leucocyte count and an automated differential were performed on EDTA blood drawn at the same time on an automated cell counter (Coulter Stacker; Coulter Electronics, Hialeah, FL).

The proportion of activated cells is expressed as a percentage of the main CD3, CD4 and CD8 cell populations or as absolute numbers (cells/μl). Background fluorescence was assessed with the appropriate isotype and fluorochrome-matched control MoAbs directed against an irrelevant target (keyhole limpet haemocyanin (KLH); Becton Dickinson). Background staining was subtracted from all the results and was always found to be $<2\%$. Intra-assay co-efficient of variation (CV) for subset analysis was always $<6\%$ and inter-assay CV $<10\%$. Median fluorescence intensity (MFI) of HLA-DR and CD25 expression was assessed only on the $CD4^+$ and $CD8^+$ lymphocyte subsets, since data were not available on the $CD3^+$ T population. MFI was measured using the FACScan Research programme (Becton Dickinson) and was expressed in arbitrary units.

Monocyte contamination of the lymphocyte gate, estimated by the percentage of $CD4^+CD3^-$ cells, was always found to be less than 0·9%. The $CD8^+$ lymphocyte population examined in this two-colour flow-cytometric technique includes both $CD3^+$ $CD8^+$ and $CD3^-CD8^+$ populations.

β_2-microglobulin and neopterin measurements

β_2-microglobulin was measured in serum using a quantitative competitive radioimmunoassay (Pharmacia AB, Uppsala, Sweden) and expressed in mg/*l*.

Neopterin was measured in serum using a quantitative competitive radioimmunoassay (Henning, Berlin, Germany) and expressed in nmol/*l*.

Statistical analysis

Differences between control subjects and HIV^+ patients in different CDC groups were compared using an analysis of variance (ANOVA) method with tests for linearity as previously described [4,5]. All variables in each test group had the normality of their distribution tested by the Kolmogorov–Smirnov goodness of fit test, and the distributions were always found to be consistent with the hypothesis of normality. Mean values for each variable in the HIV-infected subjects and in controls were compared using Student's *t*-test. Correlations were studied with Pearson's linear regression analysis.

Due to the smaller number of subjects studied for activation of the $CD4^+$ and $CD8^+$ T cell subsets, patients in groups CDC IVA and IVB–E were combined into a single group for the purpose of statistical analysis.

Frequency distributions were analysed using the χ^2 or Fisher's exact probability test for small numbers, as appropriate.

Statistical computations were performed using the Statistical Package for the Social Sciences (SPSS-X) on an IBM computer (PS/2 Model 55 SX).

RESULTS

Results are presented, first, as comparisons between all HIV^+ patients and normal control subjects, and second, between CDC groups of increasing disease severity.

Percentage levels of activated $CD3^+$ T lymphocytes

In HIV^+ patients considered as a whole, levels of $CD3^+$HLA-DR^+ T lymphocytes were higher compared with the control group ($P<0·001$) (Table 1). Levels of $CD3^+$HLA-DR^+ T lymphocytes were higher in patients in all CDC groups (II, III, IVA and IVB–E) compared with controls ($P<0·001$), but there were no differences between patients in successive CDC groups.

In contrast, levels of $CD3^+CD25^+$ T lymphocytes were significantly lower than in the control group in all HIV^+ patients considered together ($P<0·001$) (Table 1). In patients in CDC group III, percentages of $CD3^+CD25^+$ T lymphocytes were similar to control, and in CDC groups II, IVA and IVB–E significantly lower than control subjects ($P<0·001$ for all). Levels of activated $CD3^+CD25^+$ T lymphocytes were higher in patients in CDC group III and lower in CDC group IVA compared with the preceding CDC group.

Percentage levels of activated ($HLA\text{-}DR^+$ and $CD25^+$) T lymphocytes had significant tests for linear trend across the subject groups, with equally significant departure from linear trend (Table 1).

Table 1. Means (s.d.) and statistical comparison of percentages and absolute numbers of activated (HLA-DR$^+$ and CD25$^+$) T cells (CD3$^+$) and absolute numbers of CD4$^+$ T lymphocytes in subjects with HIV infection and controls

	Controls ($n=53$)	All HIV$^+$ ($n=157$)	CDC II ($n=66$)	CDC III ($n=45$)	CDC IVA ($n=10$)	CDC IVB–E ($n=36$)	Overall ANOVA ($F_{4,205}$)	Test for linear trend ($F_{1,205}$)	Departure from linear trend ($F_{3,205}$)
Per cent lymphocytes CD3$^+$HLA-DR$^+$	10·4 (5·3)	46·9† (19·3)	46·8† (19·8)	45·9†‡ (17·3)	45·8†‡ (11·3)	49·1†‡ (22·6)	46·1§	90·1§	31·4§
Per cent lymphocytes CD3$^+$CD25$^+$	14·4 (5·2)	10·2† (10·4)	9·1† (6·5)	13·9*§ (15·4)	6·9†§ (3·4)	8·4†‡ (8·8)	4·8§	5·8§	4·5§
CD3$^+$HLA-DR$^+$ lymphocytes (cells/μl)	144 (77)	633† (408)	711† (396)	711†‡ (339)	438†§ (453)	440†‡ (433)	16·5§	14·9§	17·2§
CD3$^+$CD25$^+$ lymphocytes (cells/μl)	247 (112)	119† (98)	104† (41)	183†§ (120)	68†§ (90)	74*‡ (101)	13·2§	22·9§	8·4§
CD4$^+$ lymphocytes (cells/μl)	817 (207)	349† (261)	419† (262)	417†‡ (211)	436†‡ (312)	114†§ (147)	56·0§	179·7§	14·8§

* Not significant; † $P<0·001$: these symbols refer to comparisons between HIV patient groups and controls.

‡ Not significant; § $P<0·001$: when accompanying CDC groups these symbols refer to comparisons between CDC group III, IVA, IVB–E and the preceding CDC group. When accompanying ANOVA and linear trends, they refer to comparisons across the control and CDC groups.

The $F_{4,205}$ statistic summarizes the overall analysis of all the groups. The larger its value, the greater the evidence against the null hypothesis that the group means are equivalent. $F_{1,205}$ and $F_{3,205}$ statistics should be considered together in assessing the type of trend (linear or non-linear) across the groups. Low values of both suggest no trend, whereas high values for $F_{1,205}$ and low values for $F_{3,205}$ suggest that the trend is linear.

Table 2. Means (s.d.) and statistical comparison of percentages of activated CD4$^+$ and CD8$^+$ T cell subsets (HLA-DR$^+$ and CD25$^+$) in subjects with HIV infection and control subjects

	Controls ($n=28$)	All HIV$^+$ ($n=49$)	CDC II ($n=20$)	CDC III ($n=15$)	CDC IV ($n=14$)	Overall ANOVA ($F_{3,73}$)	Test for linear trend ($F_{1,73}$)	Departure from linear trend ($F_{2,73}$)
Per cent lymphocytes CD4$^+$HLA-DR$^+$	9·7 (4·2)	26·9† (15·4)	21·4† (9·9)	25·0†‡ (15·5)	37·0†§ (17·7)	18·0§	52·3§	0·9‡
Per cent lymphocytes CD4$^+$CD25$^+$	11·0 (4·5)	29·3† (12·0)	24·0† (7·9)	31·7†§ (10·4)	34·2†‡ (15·8)	25·7§	71·4§	2·9‡
Per cent lymphocytes CD8$^+$HLA-DR$^+$	18·7 (9)	55·8† (14·5)	52·5† (13·5)	63·8†§ (12·0)	51·8†§ (15·5)	58·1§	109·6§	32·4§
Per cent lymphocytes CD8$^+$CD25$^+$	5·7 (2·9)	5·8* (5·5)	5·4* (4·7)	6·8*‡ (6·1)	5·3*‡ (6·2)	0·3‡	0·1‡	0·5‡

* Not significant; † $P<0·001$: these symbols refer to comparisons between HIV patient groups and controls.

‡ Not significant; § $P<0·001$: when accompanying CDC groups these symbols refer to comparisons between CDC group III, IV and the preceding CDC group. When accompanying ANOVA and linear trends, they refer to comparisons across the control and CDC groups.

The $F_{3,73}$ statistic summarizes the overall analysis of all the groups. The larger its value, the greater the evidence against the null hypothesis that the group means are equivalent. $F_{1,73}$ and $F_{2,73}$ statistics should be considered together in assessing the type of trend (linear or non-linear) across the groups. Low values of both suggest no trend, whereas high values for $F_{1,73}$ and low values for $F_{2,73}$ suggest that the trend is linear.

Percentage levels of activated CD4$^+$ lymphocytes

Levels of CD4$^+$ lymphocytes expressing the activation markers HLA-DR and CD25 were higher in all HIV$^+$ patients considered as a whole, and in each CDC group compared with control subjects ($P<0·001$) (Table 2, Fig. 1). There was a stepwise increase in levels of CD4$^+$HLA-DR$^+$ lymphocytes with increasing severity of disease, with significantly higher levels in CDC group IV than in the preceding CDC group ($P<0·001$). This progressive activation with disease severity showed a significant test for linear trend ($P<0·001$) without a significant departure from linear trend ($P<0·001$) (Table 2). A similar pattern was seen in levels of activated (CD25$^+$) CD4$^+$ lymphocytes, with stepwise increases with each CDC group, and a significantly higher level in CDC group III compared with CDC group II ($P<0·001$) (Table 2). Again, the test for linearity of increasing levels of activated (CD25$^+$) CD4$^+$ lymphocytes with increasing disease severity was significant ($P<0·001$), without significant departure from linearity.

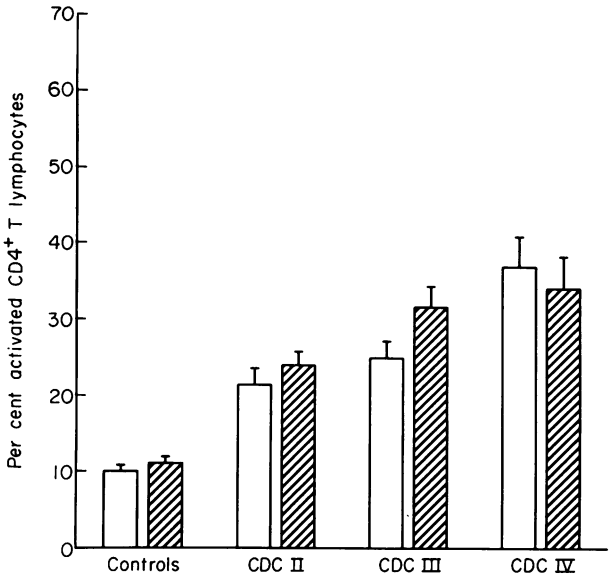

Fig. 1. Percentage levels of activated CD4+ T lymphocytes in HIV+ patients and in controls. □, CD4+ cells expressing the activation marker HLA-DR; ■, CD4+ T cells expressing the activation marker CD25. Error bars represent s.e.m.

Median fluorescence intensity of HLA-DR expression on CD4+ lymphocytes was not significantly different from control subjects in all HIV+ patients considered as a whole, and in patients in CDC groups II and III (Table 3). Median fluorescence intensity of HLA-DR expression on CD4+ lymphocytes was higher in patients in CDC IV compared with control subjects and patients in the preceding CDC group. Median fluorescence intensity of CD25 expression on CD4+ lymphocytes was higher than in control subjects in all HIV+ patients considered as a whole, and in patients in CDC groups II, III and IV ($P < 0.001$ for each). Median fluorescence intensity of CD25 expression on CD4+ lymphocytes was similar in patients in CDC II and III, but higher in patients in CDC IV compared with patients in the preceding CDC group ($P < 0.001$).

Percentage levels of activated CD8+ lymphocytes

Levels of CD8+HLA-DR+ lymphocytes were higher in all HIV+ patients compared with control subjects ($P < 0.001$) (Table 2). Levels of CD8+HLA-DR+ T lymphocytes were higher in patients in CDC groups II, III and IV compared with control subjects ($P < 0.001$ for all three). Levels were significantly higher in CDC group III and significantly lower in CDC group IV compared with the preceding CDC group ($P < 0.001$ for both).

Levels of CD8+CD25+ lymphocytes were similar in all of the disease groups and control subjects (Table 2).

Percentage levels of CD8+HLA-DR+ lymphocytes had a significant test for linearity across the subject groups and an equally significant test for departure from linear trend. There were no significant trends for linearity in levels of CD8+CD25+ lymphocytes.

MFI of HLA-DR expression on CD8+ lymphocytes was higher than in control subjects in all HIV+ patients considered as a whole, and in patients in CDC groups II, III and IV ($P < 0.001$ for each) (Table 3). There were no significant differences in MFI of HLA-DR expression on CD8+ lymphocytes between patients in CDC groups II, III and IV. MFI of CD25 expression on CD8+ lymphocytes was higher than in control subjects in all HIV+ patients considered as a whole, and in patients in CDC III and IV ($P < 0.001$ for each). There were no significant differences in MFI of expression of CD25 on CD8+ lymphocytes between patients in CDC II, III and IV.

Levels of absolute numbers of activated CD3+, CD4+ and CD8+ lymphocytes

Results obtained for absolute numbers of CD3+HLA-DR+ T lymphocytes were generally similar to those of percentages, with elevated levels in all HIV+ patients and in patients in CDC II, CDC III, CDC IVA and CDC IVB–E ($P < 0.001$ for all) compared with control subjects, and similar levels in patients in CDC groups II and III (Table 1). In contrast with percentage

Table 3. Median fluorescence intensity of HLA-DR and CD25 expression on CD4+ and CD8+ lymphocytes

	Controls ($n=28$)	All HIV+ ($n=49$)	CDC II ($n=20$)	CDC III ($n=15$)	CDC IV ($n=14$)
HLA-DR+ CD4 lymphocytes	516 (47)	526* (32)	517* (33)	508*‡ (13)	553†§ (27)
CD25+ CD4 lymphocytes	478 (28)	528† (42)	523† (45)	520†‡ (22)	540†‡ (54)
HLA-DR+ CD8 lymphocytes	590 (18)	626† (21)	626† (32)	626†‡ (13)	627†‡ (17)
CD25+ CD8 lymphocytes	586 (19)	626† (36)	614† (50)	629†‡ (11)	634†‡ (37)

Values are mean arbitrary units ± s.d.

* Not significant; † $P < 0.001$: these symbols refer to comparisons between HIV patient groups and controls.

‡ Not significant; § $P < 0.001$: when accompanying CDC groups these symbols refer to comparisons between CDC groups III, IV and the preceding CDC group.

Table 4. Means (s.d.) and statistical comparison of absolute numbers of activated CD4+ and CD8+ T cell subsets (HLA-DR+ and CD25+) in subjects with HIV infection and controls

	Controls ($n=28$)	ALL HIV+ ($n=49$)	CDC II ($n=20$)	CDC III ($n=15$)	CDC IV ($n=14$)	Overall ANOVA ($F_{3,73}$)	Test for linear trend ($F_{1,73}$)	Departure from linear trend ($F_{2,73}$)
CD4+HLA-DR+ lymphocytes (cells/μl)	68 (26)	67* (51)	76* (33)	96†§ (63)	23†§ (18)	10·3§	5·6§	12·7§
CD4+CD25+ lymphocytes (cells/μl)	82 (44)	87* (79)	90* (45)	141†§ (103)	23†§ (23)	9·8§	1·9‡	13·9§
CD8+HLA-DR+ lymphocytes (cells/μl)	88 (54)	560† (385)	579† (349)	729†§ (372)	353†§ (376)	19·8§	19·4§	19·9§
CD8+CD25+ lymphocytes (cells/μl)	26 (14)	49† (54)	49† (38)	76†§ (79)	22*§ (16)	6·1‡	0·9‡	8·7§

* Not significant; † $P<0·001$: these symbols refer to comparisons between HIV patient groups and controls.

‡ Not significant; § $P<0·001$: when accompanying CDC groups these symbols refer to comparisons between CDC group III, IV and the preceding CDC group. When accompanying ANOVA and linear trends, they refer to comparisons across the control and CDC groups.

The $F_{3,73}$ statistic summarizes the overall analysis of all the groups. The larger its value, the greater the evidence against the null hypothesis that the group means are equivalent. $F_{1,73}$ and $F_{2,73}$ statistics should be considered together in assessing the type of trend (linear or non-linear) across the groups. Low values of both suggest no trend, whereas high values for $F_{1,73}$ and low values for $F_{2,73}$ suggest that the trend is linear.

levels, however, absolute numbers of activated (HLA-DR+) CD3+ T lymphocytes were significantly lower in CDC group IVA compared with CDC group III ($P<0·001$) and remained at a similar, reduced level in patients in CDC group IVB–E. Results for absolute numbers of CD3+CD25+ T lymphocytes showed a similar pattern to those of percentage levels.

Absolute numbers of HLA-DR+ and CD25+CD3+ T lymphocytes had significant tests for linear trend and equally significant tests for departure from linear trend.

There was a slight divergence between the results obtained for absolute numbers of CD4+HLA-DR+ and CD4+CD25+ lymphocytes and the respective percentage levels, presumably reflecting changes in CD4+ lymphocyte numbers (Table 4). Compared with control subjects, absolute numbers of CD4+HLA-DR+ lymphocytes were similar in all HIV+ patients and in patients in CDC group II, higher in patients in CDC III, and lower in patients in CDC IV ($P<0·001$ for both). Numbers of CD4+HLA-DR+ lymphocytes were significantly higher in CDC group III compared with CDC group II, but significantly lower in patients in CDC group IV compared with those in CDC group III. The test for linear trend was significant, but with a much lower ($F_{1,73}$) statistic than for percentage levels and a significant departure from linear trend. An identical pattern was seen in absolute numbers of activated CD4+CD25+ lymphocytes, but in this case only the test for departure from linear trend was significant ($P<0·001$).

Levels of absolute numbers of CD8+HLA-DR+ lymphocytes had an identical distribution to percentage levels, with a significant linear trend and departure from linear trend (Table 4). Absolute numbers of CD8+CD25+ T lymphocytes, on the other hand, behaved differently to percentage levels, with higher levels in all HIV+ patients considered together and in patients in CDC groups II, III and IV compared with control subjects ($P<0·001$ for both). Numbers of CD8+CD25+ lymphocytes were higher in patients in CDC group III and lower in patients in CDC group IV than in patients in the preceding CDC group ($P<0·001$ for both).

Relationship between activation markers and CD4 count

Absolute numbers of CD4+ lymphocytes were calculated for comparative purposes, since it is the best laboratory marker of disease severity [6–8] (Table 1).

In all HIV+ patients, a significant correlation was observed between decreasing numbers of CD4+ lymphocytes and increasing percentage levels of CD3+HLA-DR+ T lymphocytes ($r=-0·52$; $P<0·001$) and between decreasing CD4+ lymphocytes and decreasing percentage levels of CD3+CD25+ T lymphocytes ($r=0·30$; $P<0·01$). No significant correlation was observed between alterations in CD4+ lymphocytes and absolute numbers of CD3+HLA-DR+ and CD3+CD25+ T lymphocytes.

A significant correlation was found between increasing percentage levels of CD4+HLA-DR+ and CD4+CD25+ lymphocytes and decreasing numbers of CD4+ lymphocytes ($r=-0·67$, $-0·53$ respectively; $P<0·001$ for both).

In all HIV+ patients a significant correlation was observed between increasing percentage levels of CD8+HLA-DR+ and decreasing numbers of CD4+ lymphocytes ($r=-0·54$, $P<0·001$).

A significant correlation was also observed in HIV+ patients between decreasing CD4+ lymphocytes and increasing absolute numbers of CD4+HLA-DR+ lymphocytes ($r=-0·54$; $P<0·001$). There was no correlation between absolute numbers of CD4+ lymphocytes and absolute numbers of CD4+CD25+, CD8+HLA-DR+ or CD8+CD25+ lymphocytes.

Relationship between cellular and soluble activation markers

In HIV+ patients, levels of β_2-microglobulin ($3·22\pm1·16$ mg/l) and neopterin ($18·8\pm4·2$ nmol/l) were increased compared with control subjects ($1·14\pm0·31$ mg/l and $6·6\pm2·2$ nmol/l respectively; $P<0·001$ for both). A positive correlation was found between increasing β_2-microglobulin levels and increasing percentage levels of CD3+HLA-DR+ T lymphocytes ($P<0·001$; $r=0·56$), but with none of the other activation subsets. No

correlation was noted between elevated neopterin levels and any of the cellular activation markers.

Comparison of frequency of abnormal levels of T cell activation markers with abnormal CD4 counts in asymptomatic HIV infection

The presence of a low CD4 count provides a marker of disease severity and risk of progression to AIDS in asymptomatic patients. In the present study abnormally low CD4 counts (less than mean −2 s.d. of control subjects) were found in 34/66 (51·5%) of patients in CDC group II. This was compared with the proportion of patients in CDC group II possessing abnormally elevated levels of $CD3^+$, $CD4^+$ and $CD8^+$ lymphocyte activation (greater than mean + 2 s.d. of control subjects).

In asymptomatic patients with HIV infection high levels of $CD3^+HLA\text{-}DR^+$ T lymphocytes were found in a greater proportion of patients (54/66; 81·8%) than abnormally low levels of $CD4^+$ lymphocytes (34/66; 51·5%) ($P < 0{\cdot}001$).

When the same analysis was performed on the smaller group of asymptomatic patients on whom CD4 and CD8 lymphocyte activation studies had been carried out, 18/20 patients (90%) had abnormally high levels of $CD8^+HLA\text{-}DR^+$ lymphocytes, compared with 12/20 (60%) having low CD4 counts, though this difference failed to reach conventional levels of statistical significance ($P = 0{\cdot}07$).

For the $CD3^+CD25^+$, $CD4^+HLA\text{-}DR^+$, $CD4^+CD25^+$ and $CD8^+CD25^+$ subsets, the frequency of abnormally high levels in asymptomatic patients was always lower than that of abnormally low CD4 counts.

DISCUSSION

In this study we demonstrate that the decline of CD4 cells in HIV infection is strongly associated with an increase in the levels of activation markers on the CD4 population, indicating a relationship between activation and death of CD4 cells. The additional observation that the levels of activation of the cytotoxic/suppressor T cell subset increase with the decline of CD4 cells may suggest a mechanism whereby the CD4 cells are eliminated. Lastly, we show that signs of cellular immune activation are present in most asymptomatic HIV-infected patients; these may prove useful early markers of disease when other indicators are still silent.

Our initial findings indicated that T lymphocyte expression of HLA-DR was persistently increased in HIV infection, and was equally high amongst all patients irrespective of CDC group. In contrast, however, when CD25 was used as an indicator of T cell activation, expression was lower in HIV-infected individuals. The dichotomy in the expression of the two activation markers prompted further studies on the expression of HLA-DR and CD25 on the $CD4^+$ and $CD8^+$ T lymphocyte subsets. The results demonstrate important differences in the activation patterns of the two T lymphocyte subsets, which may be both informative of the pathogenesis of the disease and useful clinically.

There have been previous studies of T cell activation in HIV infection, and a consistent abnormality has been an elevation of the $HLA\text{-}DR^+$ $CD8^+$ subset compared with healthy controls, a finding confirmed in the present study [9–12]. Hofmann *et al.* measured levels of CD25 expression on $CD4^+$ lymphocytes in a cohort of homosexual men at risk of HIV infection, and found higher levels amongst the HIV^- compared with the HIV^+ group, but changes in the latter patients were not related to disease severity [13]. Zola *et al.*, using a high-sensitivity immunofluorescence method, could find no difference in CD25 expression on $CD4^+$ lymphocytes in HIV-infected compared with control subjects, but the study group was small [14]. In our study the increase in the proportion of activated $HLA\text{-}DR^+$ and $CD25^+CD4^+$ lymphocytes with increasing disease severity was paralleled by an increase in the surface density of the activation markers on individual lymphocytes. The correlation between declining CD4 counts and activation of the CD4 subset adds weight to the concept that T cell activation contributes to the pathogenesis of HIV infection.

The cross-sectional nature of our study, including patients at all stages of infection, enables us only to speculate on the potency of immune markers in predicting progression of HIV-related disease. Of particular value in this respect appears to be the analysis of linearity of immune changes with increasing disease severity [4,5]. The absolute number of CD4 lymphocytes is currently the best marker of disease progression in HIV infection [15–17], and in our previous studies on the relationships between immune parameters and disease progression in HIV infection, the CD4 count, but not β_2-microglobulin, neopterin or soluble CD4, has shown a linear increase with disease severity [4]. It is of interest, therefore, that in the present study activation of the $CD4^+$ subset increases linearly with disease severity, and a longitudinal study will be required to confirm that it is indeed a useful predictor of disease progression. In addition, the close relationship between CD4 activation and CD4 decline suggests that activation of this subset may be a prerequisite for their destruction.

The power of T cell activation in predicting progression of HIV disease and its relationship to the pathogenesis of HIV disease remain to be established, and in addition, the underlying activating stimuli are not known as yet. It is possible that cell activation is secondary to the effects of HIV infection, which is known to result in increased circulating levels of cytokines such as IL-1, which is a T cell activator. However, the finding that CD8 lymphocytes do not demonstrate up-regulation of IL-2R expression in this study argues against high levels of CD25 expression on CD4 lymphocytes being solely due to the influence of cytokines. The presence of persistent activation of the $CD4^+$ cells, the major target of HIV, supports the view that cell activation is a key contributor to the pathogenesis of the disease through at least two possible mechanisms [18]. First, T cell activation involves the induction of factors which bind to specific elements, known as κB, in both the IL-2 and IL-2R genes. Similar binding sites are located in the long terminal repeat sequences of the HIV genome. Thus, activation of T cells and induction of IL-2 and IL-2R production could simultaneously result in up-regulation of synthesis of HIV mRNA [19–22]. The second possibility relates to the finding of a correlation between activated $CD8^+HLA\text{-}DR^+$ T cells and declining CD4 counts. These cells may represent the cytotoxic T cell response to HIV, which appears shortly after seroconversion [23]. Since the virus is tropic for $CD4^+$ cells, this would support the proposal by Levy *et al.* that the principal role of the CD8 cytotoxic/suppressor cell in HIV infection is to target the CD4 lymphocyte and possibly mediate its destruction [24,25].

We have also shown that the frequency of abnormally high levels of activated $HLA\text{-}DR^+$ T lymphocytes is a greater

discriminator of asymptomatic patients than a low CD4 count. This finding is of importance, since the CD4 count is frequently used to provide diagnostic information in cases of suspected immunodeficiency in which there are reasons for not performing an HIV test or the HIV test is negative. Additional diagnostic information may be gained by also measuring levels of activated T lymphocytes.

Longitudinal studies are in progress in order to determine the precise relationship of T cell activation to disease progression in individuals with HIV disease. The increase in activation of the CD8 cytotoxic/suppressor T cell subset with disease progression provides an additional possible explanation for the characteristic depletion of the CD4 cells seen in HIV infection. The linear increase in the activated helper/inducer T lymphocyte population with progressive disease may be useful in monitoring disease progression in HIV infection. Finally, our data indicate that a high level of activated HLA-DR$^+$ T lymphocytes is a more sensitive indicator of underlying immunopathology in asymptomatic HIV infection than reduced levels of CD4$^+$ cells.

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