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Association of T cell and macrophage dysfunction with surface gp120–immunoglobulin–complement complexes in HIV-infected patients

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SUMMARY

The mechanism of CD4+ cell depletion and functional T helper cell inhibition in HIV-infected individuals is poorly understood. The present study demonstrates that immune complex-covered CD4+ cells are associated with T cell inhibition and macrophage stimulation. We studied 30 patients with ARC/AIDS and 35 asymptomatic HIV+ haemophilia patients. Overall, 20±3% of peripheral CD4+ lymphocytes were covered with gp120 (range 0–94%). gp120+ cells also exhibited surface-bound IgG ($P=0.0001$), IgM ($P=0.0001$), and complement ($P=0.0001$). Decreased *in vitro* lymphocyte proliferation was associated with the immune complex load of CD4+ cells. The higher the percentage of CD4+gp120+ cells in the blood, the lower the T cell response *in vitro* ($P=0.001$). Moreover, an association was found between immune complex-positive cells and plasma neopterin ($P=0.01$). Patients with increased plasma neopterin levels had decreased *in vitro* responses to pokeweed mitogen (PWM) ($P=0.006$), phytohaemagglutinin (PHA) ($P=0.004$), concanavalin A (Con A) ($P=0.09$), and anti-CD3 MoAb ($P=0.03$), and decreased CD4+ cell counts in the blood ($P=0.006$). Since maximally 1% of CD4+ lymphocytes are infected with HIV, T cell dysfunction and T cell depletion in HIV-infected patients may also be caused by the release of free gp120 that binds to uninfected CD4+ cells. Our data suggest that the functional inhibition and subsequent elimination of uninfected CD4+ lymphocytes with surface gp120–immunoglobulin–complement complexes may be a pathomechanism in the manifestation of AIDS.

Keywords CD4+ lymphocytes HIV gp120 T lymphocyte suppression haemophilia immune complexes immunopathogenesis

INTRODUCTION

The mechanism of CD4+ cell depletion and functional T helper cell inhibition in HIV-infected individuals is poorly understood. We have shown that gp120–anti-gp120 complex-covered CD4+ lymphocytes are detectable in the peripheral blood of HIV-infected patients, and we suggested that this may be a mechanism of CD4+ cell depletion [1]. The present study demonstrates that the appearance of immune complex-covered CD4+ cells is associated with macrophage stimulation *in vivo* and T cell inhibition *in vitro*.

PATIENTS AND METHODS

Peripheral blood CD4+, CD4+gp120+, CD4+Ig+, CD4+IgG+, CD4+IgM+, and CD4+C3d+ lymphocytes, plasma neopterin levels, and *in vitro* responses of lymphocytes to stimulation with mitogens (pokeweed mitogen (PWM), phytohaemagglutinin (PHA), concanavalin A (Con A), anti-CD3 MoAb) and pooled allogeneic stimulator cells were determined in 65 HIV+ haemophilia patients. Twenty-two HIV− haemophilia patients and 88 healthy individuals served as controls. HIV+ patients were examined 1–11 times (x±s.d.=3·2±2·3). The first measurement was performed in 1988, the last in 1992. In addition to analysing the results of all tests that were performed, one measurement of each patient was chosen at random for separate statistical analysis. Of the 65 HIV+ patients, 30 patients had ARC/AIDS, and 35 were asymptomatic at the time of investigation.

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Determination of immunoglobulin, IgG, IgM, C3d and gp120 on CD4+ and CD8+ T lymphocytes

The proportion of immunoglobulin-positive CD4+ and CD8+ cells in the peripheral blood was determined using double fluorescence flow cytometry as described in detail previously [2]. Briefly, 100 μl whole blood were incubated with 10 μl anti-CD3 (OKT3, all T lymphocytes; Ortho, Raritan, NJ), anti-CD4

Table 1. Association of $CD4^+gp120^+$ cells with other $CD4^+$ cell surface markers, plasma neopterin levels, or number of impaired lymphocyte stimulation tests in 65 HIV^+ haemophilia patients

Parameter	±s.e.m.	(range)	Association of $CD4^+gp120^+$ cells with other parameters	
			r	*P*
$CD4^+gp120^+$ (%)	19·8±3·2	(0–94)		
$CD4^+IgG^+$ (%)	29·0±3·4	(0–94)	+0·594	0·0001
$CD4^+IgM^+$ (%)	58·1±4·0	(5–100)	+0·472	0·0001
$CD4^+C3d^+$ (%)	48·3±3·6	(4–99)	+0·514	0·0001
Neopterin (nmol/*l*)	26·9±1·9	(6–66)	+0·319	0·01
Number of impaired stimulation tests with PWM, Con A, PHA, anti-CD3 MoAb, or pooled allogeneic stimulator cells (RR < 0·5)	2·5±0·2	(0–5)	+0·392	0·001

PWM, Pokeweed mitogen; Con A, concanavalin A; PHA, phytohaemagglutinin; RR, relative response.

(OKT4, helper/inducer T lymphocytes; Ortho) or anti-CD8 (OKT8, suppressor/cytotoxic T lymphocytes; Ortho) MoAb for 30 min at 4°C. Erythrocytes were lysed by the addition of NH_4Cl solution for 15 min, the cells were washed with PBS, and 50 μl PE-conjugated goat anti-mouse immunoglobulin (Dianova, Hamburg, Germany) were added, together with 50 μl FITC-labelled goat anti-human immunoglobulin (Medac, Hamburg, Germany), goat anti-human IgG (Tago, Burlingame, CA), goat anti-human IgM (Medac), rabbit anti-human C3d (Dakopatts, Hamburg, Germany), or sheep anti-gp120 (Biochrom, Berlin, Germany). All antibodies were diluted 1:40. The cells were incubated for another 30 min at 4°C, washed and analysed by flow cytometry (FACScan; Becton Dickinson, Sunnyvale, CA). The gate setting for background staining was adjusted to less than 5% $CD3^+IgG^+$ control lymphocytes, and this gate was used for all subsequent analyses.

Mitogen stimulation and mixed lymphocyte culture

In vitro stimulation of lymphocytes was tested using the mitogens PWM (Gibco, Grand Island, NY), Con A (Pharmacia, Uppsala, Sweden), PHA (Wellcome, Dartford, UK), or OKT3 MoAb (Ortho). The mixed lymphocyte culture response (MLC) was assessed using allogeneic MHC-incompatible stimulator cells pooled from three healthy donors. All cultures were done in triplicate by standard methods. Relative responses (RR) were calculated as: (ct/min of patient lymphocytes cultured with mitogen − ct/min of patient lymphocytes in medium)/(ct/min of control lymphocytes cultured with mitogen − ct/min of control lymphocytes in medium). The maximum RR of each mitogen was used for statistical analysis. An RR of less than 0·5 was considered abnormally low.

Plasma neopterin

Plasma neopterin was measured with the Neopterin-RIAcid assay (Henning, Berlin, Germany). Based on control measurements in 70 healthy individuals, more than 15 nmol/*l* was considered abnormally high [3].

Statistical analysis

All data are given as mean ± s.e.m. Correlation coefficients and statistical significance were calculated using the StatView SE + Graphics program (Microsoft, Abacus Concepts, Berkeley, CA).

RESULTS

Overall, in the 65 patients studied, 20±3% of the peripheral $CD4^+$ lymphocytes were covered with gp120. The range per patient was 0–94%. These $gp120^+$ $CD4^+$ lymphocytes often had simultaneously IgG ($CD4^+gp120^+$ *versus* $CD4^+IgG^+$: $r=0·594$, $P=0·0001$), IgM ($CD4^+gp120^+$ *versus* $CD4^+IgM^+$: $r=0·472$, $P=0·0001$), and complement ($CD4^+gp120^+$ *versus* $CD4^+C3d^+$: $r=0·514$, $P=0·0001$) on their surface (Table 1).

When the patients' lymphocytes were stimulated with PWM, Con A, PHA, anti-CD3 MoAb or pooled allogeneic stimulator cells, impaired *in vitro* responses were apparent in many patients (RR of <0·5). The number of impaired test results was positively associated with the proportion of $gp120^+CD4^+$ lymphocytes ($P=0·001$) (Table 1). The higher the percentage of immune complex-positive $CD4^+$ cells, the more proliferation parameters were abnormally decreased (Fig. 1). Moreover, Con A and anti-CD3 MoAb stimulation were associated with the percentage of $CD4^+gp120^+$ lymphocytes in the blood. The higher the proportion of $CD4^+gp120^+$ cells, the lower the *in vitro* response to Con A ($r=-0·279$; $P<0·03$), PHA ($r=-0·232$; $P=0·06$), or anti-CD3 MoAb ($r=-0·273$; $P<0·05$).

An association was also seen between the fraction of immune complex-positive cells and plasma neopterin levels (Table 1). The higher the proportion of $CD4^+gp120^+$ lymphocytes, the higher the plasma neopterin levels ($P=0·01$).

These results provide evidence for a decreased T cell response and increased macrophage/monocyte activation in patients with high $CD4^+gp120^+$ cells. The macrophage/monocyte system may be stimulated *in vivo* via feedback mechanisms which become activated as a result of the decreased number of peripheral $CD4^+$ lymphocytes. That the $CD4^+$ cells are in part immune complex-covered and thereby functionally inhibited may enhance the macrophage/monocyte stimulative effect. This is supported by the finding that patients with increased plasma neopterin levels had decreased *in vitro* responses to PWM ($P=0·006$), PHA ($P=0·004$), Con A ($P=0·09$), and anti-CD3

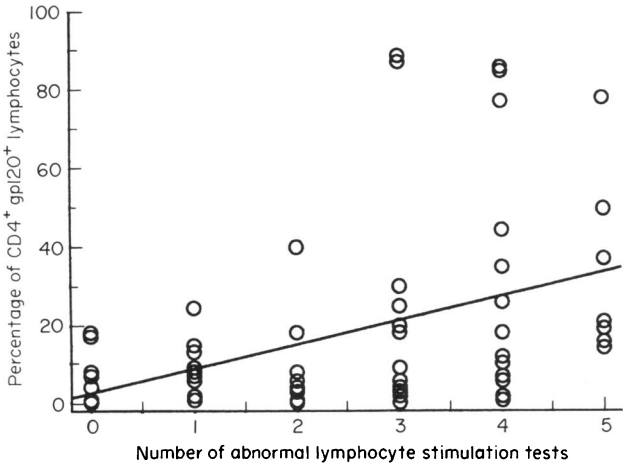

Fig. 1. Relationship of lymphocyte stimulation results with $CD4^+gp120^+$ cell counts. Lymphocytes of 65 HIV^+ patients were stimulated *in vitro* with pokeweed mitogen (PWM), phytohaemagglutinin (PHA), concanavalin A (Con A), anti-CD3 MoAb, or pooled allogeneic stimulator cells. The percentage of $CD4^+gp120^+$ lymphocytes in the peripheral blood was determined using double fluorescence flow-cytometry. Each point represents the number of impaired lymphocyte stimulation tests (relative response $<0{\cdot}5$) with the five mitogens or allogeneic cells for one patient.

MoAb ($P=0{\cdot}03$) and, moreover, decreased CD4 counts in the blood ($P=0{\cdot}006$) (Table 2).

DISCUSSION

We demonstrated recently that $CD4^+$ cell depletion in HIV-infected patients was associated with the occurrence of gp120–immunoglobulin–complement complexes on the surface of $CD4^+$ cells [1]. In the present study we obtained evidence that gp120–immunoglobulin–complement complexes on $CD4^+$ cells are associated with macrophage and T cell dysfunction.

A causal relationship between the occurrence of surface immune complexes and cell dysfunction appears likely, and we suggest the following mechanism: HIV-infected patients have periods of HIV viraemia [4–7]. If HIV mutants with high replication rates and strong CD4 affinity are generated, viraemia becomes detectable in the blood and the disease progresses [6–14]. gp120 dissociates from the viral envelope either spontaneously or after the binding of HIV to CD4 [15–20]. HIV virions but also free gp120 are bound by anti-gp120 antibodies [6,21–23] and gp120 cross-reactive anti-IgG-$F(ab)_2$ antibodies [24,25]. These complexes attach to $CD4^+$ cells, and the complement system is activated [1]. Because complex attachment is not limited to HIV-infected cells, the immune complex deposits inhibit immune functions of both infected and uninfected $CD4^+$ lymphocytes and activate macrophages [26,27], K/NK cells [28,29] and granulocytes [30].

The binding sites for gp120 and for class II MHC molecules on CD4 overlap [31]. It is likely that binding of gp120 inhibits macrophage–T helper cell interactions, induces T cell suppression, and causes macrophage activation via a negative feedback mechanism. Moreover, T cell inhibition is potentiated by autoantibodies. It was shown that MHC class II-dependent activation of resting T cells is inhibited by MoAbs to CD4, regardless of whether or not they recognize epitopes involved in the binding of MHC class II or HIV gp120 [32]. Antibodies against recombinant CD4 were found in HIV^+ patient sera [33–36], and autoantibodies against $CD4^+$ cells were associated with T helper cell defects in HIV^+ haemophilia patients [37,38].

We found impaired T cell responses in association with gp120 attachment to $CD4^+$ lymphocytes *in vivo*. In a previous study we showed that the cell separation procedure itself caused only a partial removal of autoantibodies from the lymphocyte surface [37]. Thus the lymphocytes that were isolated and cultured with mitogens and alloantigens were still covered with gp120–immunoglobulin–complement complexes. gp120 inhibits antigen-specific responses involving the T cell receptor pathway and CD4/MHC class II interaction [39]. This inhibition is reversible, and can be blocked by soluble CD4 *in vitro* [40]. Binding of gp120 decreases the CD3/T cell–antigen receptor phosphoinositide transduction pathway [41], and induces defects in Ca^{++} regulation [42]. The aberrant inositol polyphosphate metabolism reverses after azidothymidine therapy, in parallel with

Table 2. Association of plasma neopterin levels with *in vitro* responses to mitogens and $CD4^+$ blood cell counts in 65 HIV^+ haemophilia patients

Parameter	±s.e.m.	(range)	Association of neopterin with other parameters: *r*	*P*
PWM (RR)	0·7±0·1	(0–4·0)	−0·334	0·006
PHA (RR)	0·7±0·1	(0–3·3)	−0·352	0·004
Con A (RR)	0·8±0·2	(0–9·9)	−0·208	0·09
Anti-CD3 MoAb (RR)	1·9±0·4	(0–11·9)	−0·284	0·03
MLC (RR)	0·8±0·3	(0–19·3)	+0·091	0·5
Number of impaired stimulation tests with PWM, Con A, PHA, anti-CD3 MoAb, or pooled allogeneic stimulator cells (RR < 0·5)	2·5±0·2	(0–5)	+0·372	0·002

PWM, Pokeweed mitogen; PHA, phytohaemagglutinin; Con A, concanavalin A; MLC, mixed lymphocyte culture; RR, relative response.

improvements in PHA-induced proliferation responses and interferon-gamma (IFN-γ) production. Cells that bind gp120 behave as though they were chronically activated, and fail to respond to further activating signals [42]. Similar defects are seen in lymphocytes obtained from HIV-infected subjects at various stages of infection, despite the fact that only a minority of their cells are infected [42]. Short term treatment for 20 min of cells with gp120, followed by exposure to gp120 MoAb, resulted in an increase in the CD4-associated p56lck tyrosine kinase activity [43]. Long term treatment for 20 h of human T lymphocytes with gp120 resulted in the down-regulation of cell surface CD4 molecules. In addition, gp120 caused the dissociation of p56lck from CD4. However, the dissociation of p56lck from CD4 occurred at a much faster rate than the down-regulation of surface CD4 molecules [43]. gp41 and gp120 increase IL-1 and tumour necrosis factor (TNF) production and decrease IL-2, IFN-γ and IFN-α release of normal peripheral blood mononuclear cells [44], thereby inducing macrophage activation and T cell suppression. Macrophage activation was also reported by others, and is a predictive marker of disease progression [45–50]. The significant correlation between increased plasma neopterin levels, decreased lymphocyte stimulation *in vitro*, and $CD4^+$ blood cell counts observed in the present study further supports these findings.

We identified $CD4^+gp120^+$ cells in the blood, and cultured these immune-complex loaded cells with mitogens and alloantigens *in vitro*. The proportion of *in vivo* coated $CD4^+gp120^+$ cells was associated with *in vitro* lymphocyte stimulation defects. Other authors reported similar results with lymphocytes of normal volunteers coated *in vitro* with recombinant gp120. The current data provide support for the hypothesis that HIV causes T cell dysfunction and T cell depletion not only by infecting $CD4^+$ lymphocytes, but by releasing free gp120 with high CD4 affinity that binds to uninfected $CD4^+$ cells. We suggest that the functional inhibition and subsequent elimination of $CD4^+$ lymphocytes with gp120–immunoglobulin–complement complexes on the surface is an important pathomechanism of AIDS.

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REFERENCES

1 Daniel V, Süsal C, Prodeus AP *et al.* $CD4^+$ lymphocyte depletion in HIV infected patients is associated with gp120–immunoglobulin–complement attachment to $CD4^+$ cells. Vox Sang 1993; **64**:31–36.

2 Daniel V, Weimer R, Schimpf K, Opelz G. Autoantibodies against CD4- and CD8-positive T lymphocytes in HIV-infected hemophilia patients. Vox Sang 1989; **57**:172–6.

3 Schäfer AJ, Daniel V, Dreikorn K, Opelz G. Assessment of plasma neopterin in clinical kidney transplantation. Transplantation 1986; **41**:454–9.

4 Sorice F, Vullo V, Cirelli A *et al.* Blood antigens and specific anti-core and anti-envelope antibodies as markers of the course of HIV infection. Boll Ist Sieroter Milan 1989; **68**:115–21.

5 de Wolf F, Roos M, Lange JMA *et al.* Decline in $CD4^+$ cell numbers reflects increase in HIV-1 replication. AIDS Res Hum Retrovirus 1988; **4**:433–40.

6 Ellaurie M, Rubinstein A. Correlation of serum antigen and antibody concentration with clinical features in HIV infection. Arch Dis Child 1991; **66**:200–3.

7 Clark SJ, Saag MS, Decker WD *et al.* High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. N Engl J Med 1991; **324**:954–60.

8 Balfe P, Simmonds P, Ludlam CA *et al.* Concurrent evolution of human immunodeficiency virus type 1 in patients infected from the same source: rate of sequence change and low frequency of inactivating mutations. J Virol 1990; **64**:6221–33.

9 Balachandran R, Thampatty P, Enrico A *et al.* Human immunodeficiency virus isolates from asymptomatic homosexual men and from AIDS patients have distinct biologic and genetic properties. Virology 1991; **180**:229–38.

10 Shioda T, Levy JA, Cheng-Mayer C. Macrophage and T-cell tropisms of HIV-1 are determined by specific regions of the envelope gp120 gene. Nature 1991; **349**:167–9.

11 Ivey-Hoyle M, Culp JS, Chaikin MA *et al.* Envelope glycoproteins from biologically diverse isolates of immunodeficiency viruses have widely different affinities for CD4. Proc Natl Acad Sci USA 1991; **88**:512–6.

12 Cheng-Mayer C, Seto D, Levy JA. Altered host range of HIV-1 after passage through various human cell types. Virology 1991; **181**: 288–94.

13 McKeating J, Balfe P, Clapham P, Weiss RA. Recombinant CD4-selected human immunodeficiency virus type I variants with reduced gp120 affinity for CD4 and increased cell fusion capacity. J Virol 1991; **65**:4777–85.

14 Farrar GH, Roff MA, Amin T *et al.* Characterization of a series of human immunodeficiency virus isolates derived sequentially from a single patient. J Med Virol 1991; **34**:104–13.

15 Gelderblom HR, Reupke H, Pauli G. Loss of envelope antigens of HTLV-III/LAV, a factor in AIDS pathogenesis? Lancet 1985; **2**:1016–7.

16 Modrow S, Hahn BH, Shaw GM *et al.* Computer-assisted analysis of envelope protein sequence of seven human immunodeficiency virus isolates: prediction of antigenic epitopes in conserved and variable regions. J Virol 1987; **61**:570–8.

17 Layne SP, Merges MJ, Dembo M *et al.* Factors underlying spontaneous inactivation and susceptibility to neutralization of human immunodeficiency virus. Virology 1992; **189**:695–714.

18 Moore JP, McKeating JA, Weiss RA, Sattentau QJ. Dissociation of gp120 from HIV-1 virions induced by soluble CD4. Science 1990; **250**:1139–42.

19 Kirsh R, Hart TK, Ellens H *et al.* Morphometric analysis of recombinant soluble CD4-mediated release of the envelope glycoprotein gp120 from HIV-1. AIDS Res Hum Retroviruses 1990; **6**:1209–12.

20 Berger EA, Lifson JD, Eiden LE. Stimulation of glycoprotein gp120 dissociation from the envelope glycoprotein complex of human immunodeficiency virus type 1 by soluble CD4 and CD4 peptide derivatives: implications for the role of the complementarity-determining region 3-like region in membrane fusion. Proc Natl Acad Sci USA 1991; **88**:8082–6.

21 Gilbert M, Kirihara J, Mills J. Enzyme-linked immunoassay for human immunodeficiency virus type 1 envelope glycoprotein 120. J Clin Microbiol 1991; **29**:142–7.

22 Weiler BE, Schacke H, Bachmann M *et al.* Human immunodeficiency virus: novel enzyme-linked immunoassays for quantitation of envelope glycoprotein 120. J Virol Methods 1991; **32**:287–301.

23 Ellaurie M, Calvelli TA, Rubinstein A. Human immunodeficiency virus (HIV) circulating immune complexes in infected children. AIDS Res Hum Retroviruses 1990; **6**:1437–41.

24 Süsal C, Daniel V, Oberg HH *et al.* Striking inverse association of IgG-anti-Fab_γ antibodies and CD4 cell counts in patients with acquired immunodeficiency syndrome (AIDS)/AIDS-related complex. Blood 1992; **79**:954–7.

25 Süsal C, Lewin IV, Stanworth DR *et al.* Anti-IgG autoantibodies in HIV-infected hemophilia patients. Vox Sang 1992; **62**:224–9.
26 Finbloom DS, Hoover DL, Meltzer MS. Binding of recombinant HIV coat protein gp120 to human monocytes. J Immunol 1991; **146**:1316–21.
27 Dezutter-Dambuyant C, Schmitt DA, Dusserre N *et al.* Trypsin-resistant gp120 receptors are upregulated on short-term cultured human epidermal Langerhans cells. Res Virol 1991; **142**:129–38.
28 Lyerly HK, Matthews TJ, Langlois AJ *et al.* Human T-cell lymphotropic virus III_B glycoprotein (gp120) bound to CD4 determinants on normal lymphocytes and expressed by infected cells serves as target for immune attack. Proc Natl Acad Sci USA 1987; **84**:4601.
29 Weinhold KJ, Tyler DS, Lyerly HK. Measurement of direct and indirect forms of anti-HIV-1 ADCC: implications for other retroviral disease. Dev Biol Stand 1990; **72**:343–8.
30 Gabrilovich DI, Kozhich AT, Rosly IM *et al.* The synthetic peptide from HIV increases functional activity of granulocytes in healthy subjects. AIDS 1991; **5**:889–92.
31 Piatier-Tonneau D, Gastinel LN, Moussy G *et al.* Mutations in the D strand of the human CD4 V1 domain affect CD4 interactions with the human immunodeficiency virus envelope glycoprotein gp120 and HLA class II antigens similarly. Proc Natl Acad Sci USA 1991; **88**:6858–62.
32 Merkenschlager M, Buck D, Beverley PC, Sattentau QJ. Functional epitope analysis of the human CD4 molecule. The MHC class II-dependent activation of resting T cells is inhibited by monoclonal antibodies to CD4 regardless whether or not they recognize epitopes involved in the binding of MHC class II or HIV gp120. J Immunol 1990; **145**:2839–45.
33 Sekigawa I, Groopmen JE, Allan JD *et al.* Characterization of autoantibodies to the CD4 molecule in human immunodeficiency virus infection. Clin Immunol Immunopathol 1991; **58**:145–53.
34 Thiriart C, Goudsmit J, Schellekens P *et al.* Antibodies to soluble CD4 in HIV-1-infected individuals. AIDS 1988; **2**:345–51.
35 Chams V, Jouault T, Fenouillet E *et al.* Detection of anti-CD4 autoantibodies in the sera of HIV-infected patients using recombinant soluble CD4 molecules. AIDS 1988; **2**:353–61.
36 Daniel V, Weimer R, Zettlmeissl G *et al.* Autoantibodies in HIV infected hemophilia patients against different epitopes on $CD4^+$ lymphocytes and recombinant CD4. Vox Sang 1992; **62**:39–44.
37 Daniel V, Schimpf K, Opelz G. Lymphocyte autoantibodies and alloantibodies in HIV-positive haemophilia patients. Clin Exp Immunol 1989; **75**:178–83.
38 Weimer R, Daniel V, Zimmermann R *et al.* Autoantibodies against $CD4^+$ cells are associated with CD4 helper defects in human immunodeficiency virus-infected patients. Blood 1991; **77**:133–40.
39 Manca F, Newell A, Valle M *et al.* HIV-induced deletion of antigen-specific T cell function is MHC restricted. Clin Exp Immunol 1992; **87**:15–19.
40 Manca F, Habeshaw JA, Dalgleish AG. HIV envelope glycoprotein, antigen specific T-cell responses, and soluble CD4. Lancet 1990; **335**:811–5.
41 Cefai D, Debre P, Kazorek M *et al.* Human immunodeficiency virus-1 glycoproteins gp120 and gp160 specifically inhibit the CD3/T cell-antigen receptor phosphoinositide transduction pathway. J Clin Invest 1990; **86**:2117–24.
42 Nye KE, Knox KA, Pinching AJ. Lymphocytes from HIV-infected individuals show aberrant inositol polyphosphate metabolism which reverses after zidovudine therapy. AIDS 1991; **5**:413–7.
43 Juszczak RJ, Turchin H, Truneh A *et al.* Effect of human immunodeficiency virus gp120 glycoprotein on the association of the protein tyrosine kinase p56lck with CD4 in human T lymphocytes. J Biol Chem 1991; **266**:11176–83.
44 Tyring SK, Cauda R, Tumbarello M *et al.* Synthetic peptides corresponding to sequences in HIV envelope gp41 and gp120 enhance *in vitro* production of interleukin-1 and tumor necrosis factor but depress production of interferon-alpha, interferon-gamma and interleukin-2. Viral Immunol 1991; **4**:33–42.
45 Gan HX, Ruef C, Hall BF *et al.* Interleukin-6 expression in primary macrophages infected with human immunodeficiency virus-1 (HIV-1). AIDS Res Hum Retroviruses 1991; **7**:671–9.
46 Devergne O, Peuchmaur M, Humbert M *et al. In vivo* expression of IL-1 beta and IL-6 genes during viral infections in human. Eur Cytokine Netw 1991; **2**:183–94.
47 Jewett A, Giorgi JV, Bonavida B. Antibody-dependent cellular cytotoxicity against HIV-coated target cells by peripheral blood monocytes from HIV seropositive asymptomatic patients. J Immunol 1990; **145**:4065–71.
48 Fuchs D, Spira TJ, Hausen A *et al.* Neopterin as a predictive marker for disease progression in human immunodeficiency virus type 1 infection. Clin Chem 1989; **35**:1746–9.
49 Noronha IL, Daniel V, Schimpf K, Opelz G. Soluble IL-2 receptor and tumour necrosis factor-α in plasma of haemophilia patients infected with HIV. Clin Exp Immunol 1992; **87**:287–92.
50 Daniel V, Opelz G, Schäfer A *et al.* Correlation of immune defects in hemophilia with HTLV-III antibody titers. Vox Sang 1986; **51**: 35–39.