Clin Exp Immunol 1997; **107**:15–20

Escape of HIV-1 is associated with lack of V3 domain-specific antibodies *in vivo*

M. SCHREIBER, H. MÜLLER, C. WACHSMUTH, T. LAUE, F. T. HUFERT*, M. D. VAN LAER* & H. SCHMITZ *Medical Microbiology Section, Department of Virology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, and *Institute for Medical Microbiology and Hygiene, Freiburg, Germany*

(Accepted for publication 9 October 1996)

SUMMARY

This study was performed to analyse correlates of viral escape in AIDS patients. Peripheral blood mononuclear cells (PBMC) from HIV^- donors were inoculated with AIDS patients' serum to detect neutralization-resistant cell-free virus. Infectious virus was detected by polymerase chain reaction (PCR) and analysed by sequencing the V3 region. The escaped virus species was compared with all V3 virus variants found in the patients' PBMC and plasma. In one patient escaped virus was also compared with variants found in $CD4^+$ T cells isolated by FACS from blood, spleen and lymph node. The frequency of the virus variants was determined by cloning and sequence analysis of 20 V3 clones for each PCR amplification. To monitor anti-V3 antibodies by ELISA, each V3 sequence was expressed as fusion with glutathione S-transferase (GST-V3). In our AIDS patients, a V3-directed antibody response against the infectious virus V3 loop was not detectable. In contrast, virus variants unable to infect the donor PBMC *in vitro* were well recognized by homologous V3-directed antibody. After an interval of 1 year the frequency of these variants clearly decreased, while at the same time the escaped variants grew out and finally represented the predominant viral species both in plasma and PBMC. The infectious variants lacking V3 antibody response were also predominant in $CD4^+$ T cells in spleen and lymph node. Our data indicate that the escape of virus variants is closely related to the lack of V3-directed antibody.

Keywords HIV V3 loop antibody variation viral escape

INTRODUCTION

The third variable (V3) region of HIV-1 has been identified as the most variable loop-forming structure of the external glycoprotein gp120 [1,2]. Also, the V3 loop is the major target for neutralizing [3,4] and enhancing [5–7] antibodies, which are part of the immunodominant antibody response in natural and experimental HIV-1 infection [8–11].

It is generally accepted that neutralizing antibodies can be identified early in the serum of HIV-1-infected individuals [12], vaccinated humans or animals [10,13]. During progression of the disease, increasing amounts of cell-free virus (CFV) are detectable in plasma [14,15] and genetic variation promotes escape from recognition both by neutralizing antibodies and cytotoxic T lymphocytes (CTL) [16–20].

In a previous study [21] we reported that in AIDS patients the infectivity of CFV was closely related to the lack of type-specific antibodies directed against the V3 domain of the so-called iCFV. All other V3 variants simultaneously identified in the patients were unable to infect donor peripheral blood mononuclear cells (PBMC), and were therefore designated as non-iCFV. In contrast to the iCFV, these non-iCFV variants were regularly recognized by highly specific V3 antibodies. Furthermore, we demonstrated that lack of these V3-specific antibodies is the result of the continuous loss of this antibody specificity during disease progression [22].

We have now monitored the frequency changes of the iCFV, CFV and cell-integrated virus variants (CIV) over time. A detailed analysis of the variants was made in two patients. The role of the iCFV in becoming the predominant virus species in the patients was linked to the lack of iCFV-specific V3-directed antibodies.

Correspondence: Dr Michael Schreiber, Bernhard Nocht Institute for Tropical Medicine, Medical Microbiology Section, Department of Virology, Bernhard-Nocht-Str. 74, D-20359 Hamburg, Germany.

SUBJECTS AND METHODS

Subjects

Two AIDS patients, homosexual males with German nationality, were designated patient F and G. From patient F, virus variants were characterized at CDC stage IVc in 1992 and 1993. The $CD4^+$ T cell count was $< 50/\mu l$ from June 1992 until patient F died in May 1993. From patient G virus variants were characterized in 1989 and 1990. In this period of time $CD4^+$ T cell counts were $< 30/\mu l$. From patient G post-mortem material of the spleen and 10 lymph nodes was collected 3–4 h after death.

Table 1. Frequency* of V3-loop variants of patient F over time

HIV-1 variants	1992				1993			
	CIV	CFV	iCFV	Anti-V3†	CIV	CFV	iCFV	Anti-V3†
F1-01	35	<5	100	—				—
F2-05	10			—				—
F2-06	50	100		+++				+++
F2-21	5	<5		++	100	100	100	—

* Percentage of the V3-loop amino acid sequences derived from DNA sequencing of 20 independent clones; 20 of 20 = 100%.
† Autologous V3-directed antibody reactivity (—, +, ++, +++, indicating negativity and degrees of positivity).

Table 2. Frequency* of V3-loop variants of patient G over time

HIV-1 variants	1989			1990					
	CFV	iCFV	Anti-V3†	CFV	CIV	Blood	Spleen	Lymph node	Anti-V3†
G6-08	<5	80	—	75	75	65		100	—
G3-01			—		5	20			—
G7-01	40	20	—	25	15		80		—
G2-03			++		5	15			++
G2-11	40		+++						+++
G1-11			+++				20		+++
G9-01	20		+++						+++

Isolated $CD4^+$ T cells by FACS from: blood (5% HIV-1^+); spleen (5% HIV-1^+); and lymph node (12·5% HIV-1^+).
*† See Table 1.

Virus characterization

From each patient peripheral venous blood samples and citrated blood samples were taken simultaneously. Cell-free serum was obtained by two centrifugation steps (4000 ***g***, 20 min). For polymerase chain reaction (PCR) analysis patients' PBMC were isolated by Ficoll–Paque gradient centrifugation. The iCFV was isolated by culturing 1×10^6 stimulated donor PBMC (same group of donors) in 20% serum freshly collected from patients F and G. Nested PCR of cultured or patient-derived PBMC, reverse transcriptase (RT)-PCR of cell-free viral RNA, the cloning and sequencing, and the GST-V3 ELISA were carried out as described previously [21]. In brief, donor PBMC were infected *in vitro* with the patient's serum. Detection of proviral DNA was used to monitor infection. The frequency of each V3 loop variant was estimated by cloning the amplified DNA and sequencing 20 independent clones. The frequency of CIV or CFV variants was analysed by nested PCR or RT-PCR followed by the same cloning and sequencing procedure. To study the antibody response directed against the various V3 loop variants, each V3 region was cloned into the pGEX3x expression vector (Pharmacia, Freiburg, Germany) to generate a GST-V3 fusion protein. Cloning was performed by synthesis of two 109-mer oligonucleotides with 5′-GATC and TTAA-5′ overlapping ends into BamHI/EcoRI-cleaved pGEX3x. Both 109-mer oligonucleotides represent the complete coding sequence (Cys to Cys) of the V3 loop. For ELISA, affinity-purified GST-V3 fusion protein (500 ng) was applied to each well of a polystyrene 96-well tray. Human sera were tested at a dilution of 1 : 1000 and bound antibodies were detected with secondary anti-human IgG–horseradish peroxidase (HRP) conjugate.

Purification of $CD4^+$ T cells

Blood samples of patient G were collected shortly before death. $CD4^+$ lymphocytes were separated by fluorescence-activated cell sorting (FACS), as described previously [23]. $CD4^+$ lymphocytes were isolated with a purity of > 99%. Upon autopsy, lymph nodes and 50 g spleen tissue were collected. Both tissue materials were minced and digested with 0·05% collagenase dissolved in Grey's buffered salt solution (GBSS) and filtered through a 100-μm nylon gauze. After culturing, non-adherent cells were stained with anti-$CD4^+$ and anti-$CD8^+$ (Becton Dickinson, San Jose, CA), and the $CD4^+$ lymphocytes were purified using FACS (Becton Dickinson). Purified cells were tested for proviral DNA and diluted in a 10-fold dilution series to examine the amount of proviral DNA in the isolated cells. Positive PCR signals were compared with a PCR control (50 copies of the HIV-1 *env* gene, plasmid pLExHIVenv [24]). PCR-amplified proviral DNA (V3 region) representing more than 50 copies of pLExHIVenv was used for cloning and sequencing to study the frequency of virus variants.

Accession numbers

The data have been assigned the following accession numbers: Z18882 (F1-01), Z19117 (F2-05), Z19118 (F2-06), Z19119 (F2-21), Z50841 (G1-11), Z50842 (G2-03), Z50843 (G2-11), Z50844 (G3-01), Z50845 (G6-08), Z50846 (G7-01), Z50847 (G9-01).

RESULTS

V3-directed antibody reactivity against iCFV and non-iCFV variants

To identify iCFV variants, stimulated HIV-1$^-$ donor PBMC were inoculated with fresh serum samples from patients F and G. In the presence of autologous antibody, viral escape from neutralization was monitored by PCR amplification of proviral DNA. A positive signal was obtained as early as 1 day after inoculation, while directly after inoculation proviral DNA could not be detected. The amplified DNA of each isolated iCFV was cloned into pCR (Invitrogene) and 20 randomly selected V3 recombinant clones were analysed by DNA sequencing to calculate the iCFV frequency.

In patient F the V3 variant F1-01 was identified as iCFV in 1992 and F2-21 was identified as iCFV in 1993. Thus, at each time only one homogeneous virus population was isolated upon inoculating donor PBMC with serum F taken in 1992 and 1993 (20 of 20 V3 clones = 100%, Table 1). When the serum of patient G taken in 1989 was applied to donor PBMC, two iCFV variants were isolated in parallel. The frequency of these iCFV variants G6-08 and G7-01 was 80% and 20%, respectively (Table 2). Thus, in both patients two iCFV variants were replicating in competition during an interval of 1 year.

As the iCFV must be produced and released into the plasma by infected cells, we analysed the frequency of corresponding CIV variants in the infected PBMC and CFV variants in the serum. As a result of this detailed analysis four V3 variants were identified in patient F and seven in patient G (Tables 1 and 2).

The 11 V3 amino acid sequences (Fig. 1) identified in both patients were expressed as glutathione S-transferase (GST)–V3 fusion proteins to monitor the autologous V3-directed antibody response. The V3 domains of the major non-iCFV F2-06, G2-11, and G9-01 as well as the minor non-iCFV and CIV variants F2-21 (in 1992), G2-03 and G1-11 were all recognized by autologous V3 antibodies (Fig. 2). In contrast, in the serum samples used for iCFV isolation no autologous reactivity against the iCFV V3 domains were identified (F1-01, 1992; F2-21, 1993; G6-08, 1989; G7-01, 1989). Thus, the close relationship between the escape from autologous neutralization and the lack of virus-specific V3-directed antibody seems to be well documented.

Frequency changes and selection of iCFV variants over time

In Table 1 the frequencies of the iCFV, CFV and CIV variants of patient F monitored at an interval of 1 year (1992–1993) are shown. In 1992, the iCFV F2-21 variant was present at a rate of 5% in the PBMC as CIV and < 5% as CFV, thus representing a minor species. Subsequently in 1993, when isolated as iCFV, F2-21 was predominating both as CFV (100%) and CIV (100%). Thus, during 1 year iCFV F2-21 became totally predominant in the blood. In contrast, non-iCFV F2-06 presenting 100% of the CFV in 1992 disappeared within 1 year. Also F1-01, the iCFV isolated in 1992, was no longer detected. In conclusion, both iCFV variants must have circulated simultaneously in the blood, but F2-21 finally had overgrown F1-01.

For patient G, frequency changes of iCFV variants G6-08 and G7-01 over 1 year are shown in Table 2. In this patient the frequency of iCFV V3 variants was followed not only in serum (CFV) and PBMC (CIV), but also in FACS-purified CD4$^+$ T cells from blood, spleen and lymph nodes. Blood samples were taken shortly before death and tissue material was obtained post-mortem.

```
BH-10    CTRPNNNTRK.SIRIQRGPGRAFVTIGKI.GNMRQAHC

F1-01    -------I-IRH-H-..------HATEAAT-DI-----
F2-05    -------V-IRH-H-..------YATEA-T-DI-----
F2-06    ----S---S-.G-H-..------YATEA-T-DI-----
F2-21    ----S---S-.G-H-..------HATEAAT-DI-----

G6-08    ----------.--P-..------Y-TRQ-I-DI-----
G3-01    ----------.G-P-..------Y-TKQ-I-DI-----
G7-01    --------G-.R-S-..----S-Y-TRQ-V-DI-----
G2-03    --------G-.R-S-..------Y-TRQ-I-DI-----
G2-11    --------G-.R-S-..----S-Y-TRQ-I-DI-----
G1-11    ----------.R-S-..----S-Y-TRQ-V-DI-----
G9-01    --------G-.--S-..------Y-TRQ-I-DI-----
```

Fig. 1. Amino acid sequences of V3 loop variants detected in patients F and G. Each V3 loop sequence is aligned to the HIV-1 BH-10 V3 sequence. Dashes indicate identical amino acids. Dots indicate gaps introduced to improve the alignment.

In 1989, variant G6-08 was the major iCFV (80%), but with a frequency of < 5% among the CFV in serum. In addition to G6-08, a second minor iCFV (20%) was found contributing to the CFV by 40%. One year later (1990), both iCFV variants were prevailing as CIV in the PBMC and as CFV in serum. In contrast, the non-iCFV variants G2-11 and G9-01 could no longer be detected. Two new variants, G3-01 and G2-03, were detected in PBMC in 1990, but at a frequency of only 5%. Thus, during 1 year a complete turnover of the CFV species was observed. The frequency of V3 variants recognized by V3 antibodies was clearly reduced, while the frequency of the iCFV had increased significantly.

Moreover, FACS-purified CD4$^+$ T cells from blood, spleen, and lymph nodes were analysed for HIV-1 proviral DNA, showing high infection rates of 5%, 5% and 12·5%, respectively. When the proviral DNA was cloned and sequenced, it turned out that both iCFV variants G6-08 and G7-01 were predominant. G6-08 represented not only the majority of CFV and CIV in 1990, it also made up 65% and 100% of the variants in infected blood CD4$^+$ and lymph node CD4$^+$ T cells, respectively. The second iCFV, G7-01,

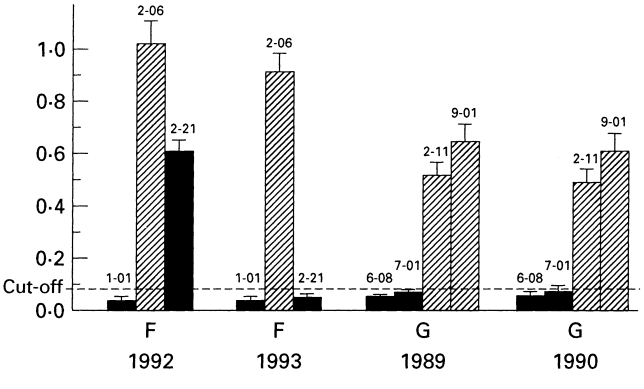

Fig. 2. Autologous antibody reactivities directed against iCFV and the major non-iCFV V3 loops in the sera of patients F and G as measured by ELISA. For each test 500 ng of the affinity chromatography-purified glutathione S-transferase (GST)–V3 fusion protein, equivalent to 50 ng of V3 peptide, were applied to each well of a 96-well microtitre plate. ■, Reactivities directed against the iCFV V3 loop variants; ▨, reactivities directed against the non-iCFV V3 loops detected as the variants which represent most of the CFV in patients' serum (F, 1992; G, 1989). The black line indicates the cut-off level calculated from a pool of HIV-1$^-$ sera tested. Representative of four experiments.

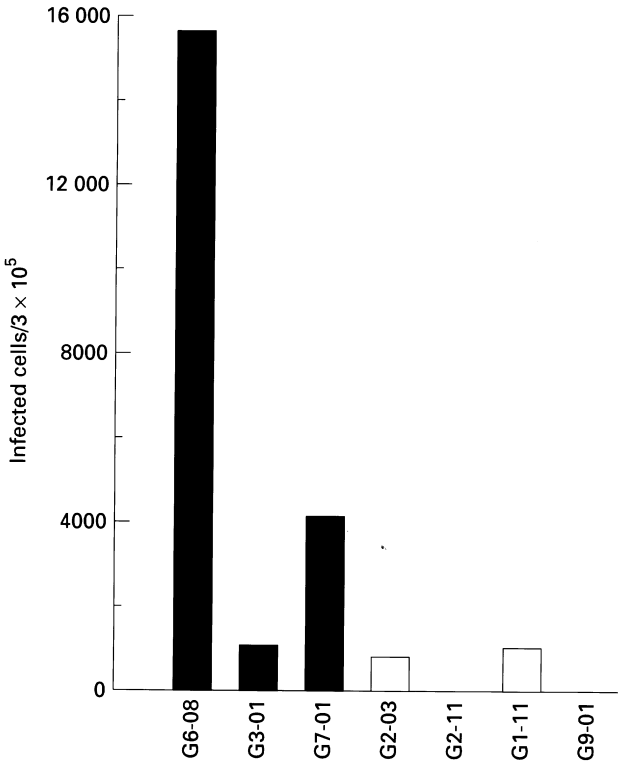

Fig. 3. Frequency of iCFV and non-iCFV V3 variants in FACS-sorted $CD4^+$ T cells of patient G. ■, V3 loop variants which are not recognized by autologous anti-V3 loop antibodies. These variants represent 92% of the infected $CD4^+$ T cells present in blood, spleen, and lymph nodes. □, The V3 loop variants (8%) which were well recognized by autologous serum. For calculation, the frequency of a V3 variant from Table 2 together with the number of HIV-1 copies per 10^5 cells (percent of infection $\times 10^3$) gives the amount of cells infected by this V3 variant.

was predominant in spleen $CD4^+$ T cells (80%) and was also present at a high rate in the CFV (25%) and CIV (15%) species. In contrast, both major non-iCFV variants G2-11 and G9-01, contributing to CFV by 60% in 1989, were not detectable in the blood or purified $CD4^+$ T cells 1 year later. V3 variants G2-03 and G1-11, well recognized by serum of patient G, represented only a minority in purified $CD4^+$ T cells.

From the frequency data in Table 2 and the infection rates of each T cell fraction (blood 5%, spleen 5%, lymph node 12·5%) the frequencies of each variant in 10^6 purified $CD4^+$ T cells can be calculated. As shown in Fig. 3, the variants recognized by autologous serum (G2-03, G2-11, G1-11, G9-01) represented only 8% of the provirus found in the FACS-purified HIV-1-infected $CD4^+$ T cells. In contrast, the predominant species in these cells were iCFV variants G6-08 and G7-01, followed by G3-01. Altogether, the variants which were not recognized by V3-directed antibodies were predominant in this T cell population by 92% (Fig. 3).

Our data show that certain iCFV variants lacking type-specific V3 antibody became predominant not only as part of the CFV species in plasma, but also in infected $CD4^+$ T cells from blood, spleen and lymph node.

DISCUSSION

Escape of HIV-1 from humoral immune response is well documented. It was assumed that escape might be due to extensive viral replication, leading to mutants no longer recognized by the immune system [18,25,26]. In keeping this theory is the close relationship between the outgrowth of certain virus variants and the lack of the corresponding V3-specific antibody. Apart from the iCFV, we could not isolate any other cell-free variants, suggesting that the suppression of these variants was due to the presence of the type-specific V3-directed antibody. Since neutralizing V3-directed antibodies have been described in HIV-1-infected subjects, their function in disease progression is controversial [27,28]. In part, the mapping of anti-V3 antibodies to short synthetic peptides which partially imitate the V3 loop structure might account for controversial results. To avoid this problem, our experimental design was based on strictly autologous experiments and full-length V3 loops (Cys to Cys) expressed and purified from *Esherichia coli*. When full-length V3 loop sequences were used for the detection of autologous sera, a high specificity of antibody response was observed. This high specificity was not observed with short peptides spanning the identical V3 sequences (pep-scan analysis, data not shown). Thus the synthesis of the full-length V3 loop is needed to detect differences in the V3 antibody response [21,22]. In humans, progression of the disease was shown to correlate with *env*-directed or V3-directed homologous antibodies [29] or with loss of neutralizing antibody titres [30–32]. Using our GST–V3 fusion proteins we could detect the loss of V3-specific antibodies during progression of the disease in correlation to the emergence of the iCFV [22]. Here we observed a similar loss of V3-specific antibody directed against the iCFV V3 loop F2-21. This variant appeared after the F2-21 V3-specific antibody had disappeared from the serum of patient F, suggesting that besides antigenic variation, the loss of antibody also contributes to viral escape and selection.

In both patients F and G, two iCFV variants without corresponding V3-directed antibody were replicating in parallel over an interval of 1 year. Despite the high multiplication rates of HIV-1 [33,34], V3 variants with identical sequences can be detected over a period of more than 3 years in plasma or PBMC [35]. Also, identical V3 sequences were found in epidemiologically unlinked patients [36], suggesting that the V3 loop reflects mainly the selective forces of the immune system and the need to infect a variety of cell types which are characterized by HIV-1-specific cofactors [37,38]. Our observation on the outgrowth of F2-21 and G6-08 in competition with other iCFV variants suggests that additional anti-viral factors like V2-directed antibodies or variant-specific CTL are responsible for controlling iCFV replication. We are aware of the fact that our observation is confined only to the interaction of CFV and PBMC, while the role of anti-CFV V3 antibody in the infection of macrophages or dendritic cells needs further study. Nevertheless, the detailed analysis of $CD4^+$ T cells from blood, spleen, and lymph node additionally demonstrates the *in vivo* relevance of iCFV as the predominant T cell trophic variant at the final state of the disease.

Although sufficient iCFV V3 antigen must have been produced in both patients, no production of the corresponding antibody was seen after 1 year. This may be due to the progression of the immune suppression of both AIDS patients. The low level of $CD4^+$ T helper cells may have prevented the induction of the V3-specific humoral immune responses [39].

Our data on the close relationship between lack of type-specific V3-directed antibody, the escape, and outgrowth of certain variants in AIDS patients suggest that type-specific V3-directed antibodies

are an important marker in analysing autologous neutralization, and might be responsible for the control of cell-free HIV-1 *in vivo*.

ACKNOWLEDGMENTS

We thank N. Thum-Schmitz and A. Seifert for technical assistance. This work was supported by the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie to M.S. and H.S. (BMBF, FKZ:01K19469/B), and a Wellcome Prize Fellowship to C.W. This work counts in partial fulfilment of the PhD requirements for C.W. and H.M. at Hamburg and Bremen University.

REFERENCES

1 LaRosa GJ, Davide JP, Weinehold K *et al.* Conserved sequence and structural elements in the HIV-1 principle neutralizing determinant. Science 1990; **249**:932–5.

2 Moore JP, McCutchan FE, Poon SW *et al.* Exploration of antigenic variation in gp120 from clades A through F of human immunodeficiency virus type 1 by using monoclonal antibodies. J Virol 1994; **68**:8350–64.

3 Moore JP, Trkola A, Korber B *et al.* A human monoclonal antibody to a complex epitope in the V3 region of gp120 of human immunodeficiency virus type 1 has broad reactivity within and outside clade B. J Virol 1995; **69**:122–30.

4 Spear GT, Takefman DM, Sharpe S, Ghassemi M, Zolla Pazner S. Antibodies to the HIV-1 V3 loop in serum from infected persons contribute a major proportion of immune effector functions including complement activation, antibody binding, and neutralization. Virology 1994; **204**:609–15.

5 Homsy J, Meyer M, Levy JA. Serum enhancement of human immunodeficiency virus (HIV) correlates with disease in HIV-infected individuals. J Virol 1990; **64**:1437–40.

6 Jiang SB, Lin K, Neurath AR. Enhancement of human immunodeficiency virus type 1 infection by antisera to peptides from the envelope glycoproteins gp120/gp41. J Exp Med 1991; **174**:1557–63.

7 Kliks SC, Shioda T, Haigwood NL, Levy JA. V3 variability can influence the ability of an antibody to neutralize or enhance infection by diverse strains of human immunodeficiency virus type 1. Proc Natl Acad Sci USA 1993; **90**:11518–22.

8 Carrow EW, Vujcic LK, Glass WL *et al.* High prevalence of antibodies to the gp120 V3 region principal neutralizing determinant of HIV-1 MN in sera from Africa and the Americas. AIDS Res Hum Retrovir 1991; **7**:831–8.

9 Zwart G, Langedijk H, van der Hoek L *et al.* Immunodominance and antigenic variation of the principle neutralization domain of HIV-1. Virology 1991; **181**:481–9.

10 Berman PW, Groopman JE, Gregory T *et al.* HIV-1 challenge of chimpanzees immunized with recombinant gp120. Proc Natl Acad Sci USA 1988; **85**:5200–4.

11 Goudsmit J, Juiken CL, Nara PL. Linear *versus* conformational variation of V3 neutralization domains of HIV-1 during experimental and natural infection. AIDS 1989; **3**:S119–23.

12 Moore JP, Cao Y, Ho DD, Koup RA. Development of the anti-gp120 antibody response during seroconversion to human immunodeficiency virus type 1. J Virol 1994; **68**:5142–55.

13 Girard M, Kieny MP, Renter A *et al.* Immunization of chimpanzees confers protection against challenge with human immunodeficiency virus. Proc Natl Acad Sci USA 1991; **88**:542–6.

14 Piatak M, Saag MS Jr, Yang LC *et al.* High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science 1993; **259**:1749–54.

15 Schnittman SM, Greenhouse JJ, Lane HC, Pierce PF, Fauci AS. Frequent detection of HIV-1-specific mRNAs in infected individuals suggests ongoing active viral expression in all stages of disease. AIDS Res Hum Retrovir 1991; **7**:361–7.

16 Albert J, Abrahamsson B, Nagy K *et al.* Rapid development of isolate-specific neutralizing antibodies after primary HIV-1 infection and consequent emergence of virus variants which resist neutralization by autologous sera. AIDS 1990; **4**:107–12.

17 di Marzo Veronese F, Reitz MS, Gupta G Jr *et al.* Loss of a neutralizing epitope by a spontaneous point mutation in the V3 loop of HIV-1 isolated from an infected laboratory worker. J Biol Chem 1993; **268**:25894–901.

18 McKeating JA, Bennett J, Zolla Pazner S *et al.* Resistance of a human serum-selected human immunodeficiency virus type 1 escape mutant to neutralization by CD4 binding site monoclonal antibodies is conferred by a single amino acid change in gp120. J Virol 1993; **67**:5216–25.

19 Pircher H, Moskophidis D, Rohrer U, Burki K, Hengartner H, Zinkernagel RM. Viral escape by selection of cytotoxic T cell-resistant virus variants *in vivo*. Nature 1990; **346**:629–33.

20 von Gegerfelt A, Albert J, Morfeldt-Manson L, Broliden K, Fenyo EM. Isolate-specific neutralizing antibodies in patients with progressive HIV-1-related disease. Virology 1991; **185**:162–8.

21 Schreiber M, Petersen H, Wachsmuth C, Müller H, Hufert F, Schmitz H. Antibodies of symptomatic human immunodeficiency virus type 1-infected individuals are directed to the V3 domain of noninfectious and not of infectious virions present in autologous serum. J Virol 1994; **68**:3908–16.

22 Schreiber M, Wachsmuth C, Müller H, Hagen C, Schmitz H, van Lunzen J. Loss of antibody reactivity directed against the V3 domain of certain human immunodeficiency virus type 1 variants during disease progression. J Gen Virol 1996; **77**:2403–14.

23 von Laer D, Hufert F, Fenner TE *et al.* $CD34^+$ hematopoietic progenitor cells are not a major reservoir of the human immunodeficiency virus. Blood 1990; **76**:1281–6.

24 Bosch V, Pawlita M. Mutational analysis of the human immunodeficiency virus type 1 env gene product proteolytic cleavage site. J Virol 1990; **64**:2337–44.

25 Nara PL, Smit L, Dunlop N *et al.* Emergency of viruses resistant to neutralization by V3-specific antibodies in experimental human immunodeficiency virus type 1 IIIB infection of chimpanzees. J Virol 1990; **64**:3779–91.

26 Reitz MS, Wilson C, Naugle C, Gallo RC, Robert Guroff M. Generation of a neutralization-resistant variant of HIV-1 is due to selection for a point mutation in the envelope gene. Cell 1988; **54**:57–63.

27 Ariyoshi K, Harwood E, Chiengsong Popov R, Weber J. Is clearance of HIV-1 viraemia at seroconversion mediated by neutralising antibodies? Lancet 1992; **340**:1257–8.

28 Robert Guroff M, Roilides E, Muldoon R *et al.* Human immunodeficiency virus (HIV) type 1 strain MN neutralizing antibody in HIV-infected children: correlation with clinical status and prognostic value. J Infect Dis 1993; **167**:538–46.

29 Pincus SH, Messer KG, Nara PL, Blattner WA, Colclough G, Reitz M. Temporal analysis of the antibody response to HIV envelope protein in HIV-infected laboratory workers. J Clin Invest 1994; **93**:2505–13.

30 Lu W, Shih WK, Tourani JM, Eme D, Alter HJ, Andrieu JM. Lack of isolate-specific neutralizing activity is correlated with an increased viral burden in rapidly progressing HIV-1-infected patients. AIDS 1993; **7**:S91–99.

31 Cavacini LA, Emes CL, Power J *et al.* Loss of serum antibodies to a conformational epitope of HIV-1/gp120 identified by a human monoclonal antibody is associated with disease progression. J Acquir Immune Defic Syndr 1993; **6**:1093–102.

32 Fenouillet E, Blanes N, Benjouad A, Gluckmann JC. Anti-V3 antibody reactivity correlates with clinical stage of HIV-1 infection and with serum neutralizing activity. Clin Exp Immunol 1995; **99**:419–24.

33 Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 1995; **373**:123–6.

34 Wei X, Ghosh SK, Taylor ME *et al.* Viral dynamics in human immunodeficiency virus type 1 infection. Nature 1995; **373**:117–22.

35 Simmonds P, Zhang LQ, McOmish F, Balfe P, Ludlam CA, Leigh

Brown AJ. Discontinuous sequence change of human immunodeficiency virus (HIV) type 1 env sequences in plasma viral and lymphocyte-associated proviral populations *in vivo*: implications for models of HIV pathogenesis. J Virol 1991; **65**:6266–76.,

36 Holmes EC, Zhang LQ, Robertson P *et al.* The molecular epidemiology of human immunodeficiency virus type 1 in Edinburgh. J Inf Dis 1995; **171**:45–53.

37 Feng Y, Broder CC, Kennedy PE, Berger EA. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane domain, G-protein coupled receptor. Science 1996; **272**:872–7.

38 Alkhatib G, Combadiere C, Broder CC *et al.* CC CKR5: a rantes, MIP1α, MIP-1β receptor as a fusion cofactor for macrophage-tropic HIV-1. Science 1996; **272**:1955–8.

39 Miedema ML, Petit AJC, Terpstra FG *et al.* Immunological abnormalities in human immunodeficiency virus (HIV)-infected asymptomatic homosexual men. J Clin Invest 1988; **82**:1908–14.