Loss of antibody reactivity directed against the V3 domain of certain human immunodeficiency virus type 1 variants during disease progression

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We have previously shown that in AIDS patients a predominant species of infectious virus can be found which is not neutralized by homologous serum. The presence of the infectious virus was associated with the lack of type-specific antibody directed against the V3 domains of these virions. In contrast to this lack of V3-specific antibody, the other V3 domains of non-infectious virions were well recognized by antibody. To determine whether the lack of a V3-specific antibody response is due to a progressive loss of antibody during human immunodeficiency virus type 1 (HIV-1) infection, we monitored the anti-V3 antibody response in 90 patients over time. Anti-V3 antibodies were monitored by a V3-specific ELISA using 21 different V3 domains as a fusion with glutathione S-transferase (GST-V3) based upon sequences from 11 HIV-1 patient isolates and 10 sequences from an HIV-1 B subtype consensus-like GST-V3 expression library. This strictly heterologous screening showed a loss of V3-specific antibodies in 20 out of the 90 patients tested. To study the in vivo relevance of these findings we analysed V3 antibody loss in two patients. This strictly autologous antibody screening was performed based upon V3 sequences of the patients' cell-free virions. In both patients the loss of a V3-specific antibody could be detected in parallel to a decline of CD4+ T cells. Moreover, the escape of a distinct V3 variant was shown to correlate closely with the loss of the V3-specific antibody.

Introduction

The V3 domain of human immunodeficiency virus type 1 (HIV-1) gp120 glycoprotein is the target of the immunodominant antibody response in natural and experimental HIV-1 infection (Berman et al., 1988; Goudsmit et al., 1988, 1989; Carrow et al., 1991; Nara et al., 1990; Zwart et al., 1991; Moore et al., 1994). Antibodies directed against the V3 domain can have neutralizing (Spear et al., 1994) as well as enhancing activity (Homsy et al., 1990; Jiang et al., 1991; Kliks et al., 1993). This may be one reason for the controversy over the role of V3 antibody in controlling HIV-1 in vivo (Girard et al., 1991, Montefiori et al., 1992). Also, studies correlating heterologous or autologous V3-antibody reactivity to disease progression have generated contradictory results (Hogervorst et al., 1995; Moore et al., 1996; Kostrikis et al., 1996).

However, it is generally accepted that under selective pressure, virus variation leads to escape variants which are no longer controlled by HIV-1-specific cytotoxic T lymphocytes (CTL) (Pircher et al., 1990), antibody (Albert et al., 1990; von Gegerfeldt et al., 1991) or antiviral drugs (Ho et al., 1995; Wei et al., 1995). As a result of viral escape, increasing amounts of cell-free virus (CFV) are detectable in plasma during the progression of the disease (Piatak et al., 1993). This increase of the viral load parallels the concomitant increase of variability from at least 10^6 variants in asymptomatic patients to more than 10^8 genetically distinct virus variants in AIDS patients. As
a result, viral escape mutants can be isolated more frequently from the plasma of AIDS patients (Coombs et al., 1989). Moreover, drug-resistant mutations may be present among these variants, even in the absence of the selective pressure induced by drug therapy (Najera et al., 1995).

The development of a more heterogeneous viral quasi-species has been shown to be associated with a concomitant decline of CD4+ T cells and AIDS progression (Strunnikova et al., 1995). In addition to the progressive CD4+ T cell decline during HIV-1 infection (Rosenberg & Fauci, 1988) the lack of neutralizing serum antibodies directed against the V3 domain (Page et al., 1992; Lu et al., 1993; Fenouillet et al., 1995) or directed against a conformational epitope of the gp120 CD4-binding region (Cavacini et al., 1993) was identified in association with disease progression.

In a previous report we demonstrated that antibodies of symptomatic HIV-1-infected individuals are directed against the V3 domains of the non-infectious virions and not against the infectious CFV (iCFV) which could be isolated from the patients' serum. Thus the infectivity of certain cell-free virions was shown to be closely related to the lack of type-specific antibodies directed against the so-called iCFV V3 domain (Schreiber et al., 1994).

We have now monitored the V3-specific antibody reactivity over time to test the hypothesis that the loss of a V3-specific antibody serves as a possible mechanism for viral escape. The experimental design of the antibody screening to show the V3 antibody loss in a large group of patients (90) was straightforward. Patients were randomly selected based on the availability of serum samples over a period of 4 years only. The antibody screening was strictly heterologous and two different sets of V3 sequences were used for the detection of V3-specific antibodies in the patients' sera. One set was derived from sequences of patient isolates, the so-called PAT V3 domains. The other set of sequences was obtained from a HIV-1 B subtype consensus-like V3 expression library, the so-called SYN V3 domains.

In a strictly autologous analysis of the anti-V3 antibody reactivity in two patients we confirmed our observation of a V3-specific antibody loss. A continuous antibody decline was found in both patients closely related to the release of a corresponding iCFV. Also, the loss of antibody was paralleled by the decline of CD4+ T cells in both patients.

Because of the detailed analysis of the anti-V3 antibody response over time, we provide evidence for a hitherto unknown mechanism supporting viral escape of a distinct V3 virus variant. This mechanism is the progressive loss of the corresponding HIV-1 V3-specific antibody.

**Methods**

**Subjects.** Serum samples from 90 HIV-1-infected German individuals (A1, A2, A3, ... , B1, B2, B3, ... etc.) had been collected at annual intervals and had been stored at −70 °C. Also CD4+ T cell counts had been monitored by routine fluorescence-activated cell sorting (FACS) analysis. Eighteen of the 90 subjects revealed stable numbers of CD4+ T cells within a range of 800–500 cells/μl. Nineteen subjects had similar numbers of CD4+ T cells at the time of the first serum sample but had negative regression slopes. These 19 patients had more than 200 CD4+ T cells/μl in the last year of serum sampling. In all the other 53 patients the CD4+ T cell count was finally below 200 cells/μl (20 patients, 200–100 cells/μl; 33 patients, < 100 cells/μl).

A second pool of sera was obtained from 40 HIV-1-infected individuals who were asymptomatic and lived in Hamburg, Germany. This pool was used to select antibody-reactive V3 loop-expressing bacterial clones only.

AIDS patients C and F were both homosexual males of German origin. From patient C serum was first available in 1/87 and the CD4+ T cell count was 600/μl. A decline of CD4+ T cells was observed over time and numbers got below 200/μl in 1/89. In 12/89 the CD4+ T cell count was below 30/μl and was further declining until patient C died in 8/93. From patient F serum was first available in 1/88 and the CD4+ T cell count was 800/μl. A continuous decline was monitored and CD4+ T cell count fell below 200/μl in 1/90 and < 50/μl from 6/92 until patient F died in 5/93. Virus variants from both patients were characterized in 1992.

**iCFV and non-iCFV characterization.** The iCFV isolation from serum and the cloning and sequencing of the V3 region has been described before (Schreiber et al., 1994). In brief, to characterize the iCFV of each patient 1 ml fresh serum was incubated with 4 ml culture medium [RPMI, 15% fetal calf serum (FCS)] containing 2 × 10^6 prestimulated donor PBMC. Donor PBMC were obtained from a known HIV-1-responding individual by Ficol-Paque gradient centrifugation and were prestimulated with phytohemagglutinin (PHA) and interleukin-2 (IL-2) (25 U/ml) for 3 days. After PHA and IL-2 activation, one-fifth of the donor PBMC culture medium was removed and 1 ml of fresh serum from the HIV-1 patient was added. On days 1, 3, 5 and 7 a sample of 1 ml was harvested by centrifugation and monitored for the presence of proviral DNA by nested PCR. The frequency of the V3 sequences was determined by cloning of the amplified DNA into pcRII and sequencing of 20 clones. To identify the non-iCFV variants, viral RNA from plasma was isolated from 200 μl of serum by binding to acid-washed silica beads (0.5–10 μm) and was eluted in 50 μl of RNase-free water (56 °C, 5 min). Ten μl was used for reverse transcription with 200 U of Moloney murine leukaemia virus reverse transcriptase (37 °C, 1 h). The cDNA/RNA hybrids were denatured (95 °C, 10 min) and used for PCR as described. The frequency of the non-iCFV was also determined by cloning and sequencing of 20 clones.

**Expression of glutathione S-transferase (GST)–V3 fusion proteins.** To study the antibody response of HIV-1-infected individuals to different V3 domains, each V3 region was cloned into the pGEX3x expression vector (Pharmacia) to generate GST–V3 fusion proteins. For each V3 variant two 109-mer oligonucleotides were synthesized and hybridized to form a double-stranded DNA fragment with 5′ GATC, CCCAATAACAATACAAGAAAA(AG)G(AT)ATAC(AC)TATAGGG-TTAA 5′ overlapping ends. This fragment represents the complete V3 loop-coding sequence (Cys to Cys) and was cloned into BamHI/EcoRI sites of the pGEX3x expression vector. The plasmid was linearized with S′ GATC, TTAA 5′ overlapping ends. This fragment represents the complete V3 loop-coding sequence (Cys to Cys) and was cloned into BamHI/EcoRI-cleaved pGEX3x. After DNA sequencing GST–V3 expression was tested in small-scale culture.

**Generation of the HIV-1 B subtype V3 loop library.** A large number of V3 loop variants of the B subtype V3 consensus sequence was synthesized by cloning of a synthetic degenerate DNA fragment (variability: 211 = 2048) into the BamHI and EcoRI sites of the pGEX3x expression vector. To obtain a dsDNA fragment two 109-mer oligonucleotides were synthesized and hybridized (S′ GATCTGTACACAGA-CCCCAATACAATACAAGAAAA(AG)GATATAC(AC)TATAGGG-CCCGGAGAGCCTTTT(CT)AT(AG)CTACT(AG)GAG(AG)(AG)CT-
V3-specific ELISA. Bacterial cultures (OD_{600} = 0.7) were induced by adding IPTG to a final concentration of 0.1 mM. Bacterial growth was continued for 5 h and the bacteria were lysed by sonication at 0 °C in GST buffer (150 mM-NaCl, 16 mM-Na_2HPO_4, 4 mM-NaH_2PO_4, 1% Triton X-100, pH 7.5). Cleared extracts were applied to glutathione–agarose beads (Sigma) and the bound GST–V3 protein was eluted with 5 mM-glutathione in 50 mM-Tris-HCl, pH 7.5. Purified GST–V3 was analysed on an SDS/15% polyacrylamide gel. Only a single band of the GST–V3 protein was seen after Coomassie staining without background staining of contaminating Escherichia coli proteins. Also, no background or false-positive signals could be detected by Western blot using a pool of negative control sera. For ELISA, 500 ng of each GST–V3 was applied to each well of a polystyrene 96-well tray (MaxiSorb, Nunc). Human sera were tested at a dilution of 1:1000. Bound antibodies were detected with secondary goat anti-human IgG–horseradish peroxidase (HRP) conjugate (Bio-Rad; 1:1000, 100 µl each well, 1 h, 37 °C) as described (Schreiber et al., 1994). The cutoff level was determined by using a pool of 20 HIV-1-negative sera.

Western blotting. For anti-HIV-1 antibody testing commercial Western blot strips were used (NovaPath HIV-1 Immunoblot, Bio-Rad). Strips were blocked with in MNTT buffer (10% low-fat milk, 100 mM-NaCl, 10 mM-Tris–HCl, 0.05% Tween 20, pH 7.5) for 30 min. Serum samples were diluted 1:100 in MNTT buffer and exposed to the Western blot strips for 2 h. Bound antibody was detected with secondary anti-human IgG–HRP-conjugated antibodies (Bio-Rad; 1:750 in MNTT). Strips were washed twice in NTT (100 mM-NaCl, 10 mM-Tris–HCl, 0.05% Tween 20, pH 7.5) and NT (100 mM-NaCl, 10 mM-Tris–HCl, pH 7.5) buffer and stained using 4-chloro-1-napththol (Sigma) as HRP substrate.

Results

Loss of heterologous V3-specific antibodies

To obtain information on the loss of V3 antibodies during HIV-1 infection we screened paired serum samples collected over an interval of at least 4 years from 90 HIV-1-infected individuals for their reactivity against different V3 domains. To generate various B subtype V3 domains, mixed amino acids at positions 306 (G, S, R), 308 (P, H), 316 (Y, H), 317 (T, A), 319 (G, E, R, K), 320 (E, D, K, N, A, T), and 324 (D, E) were introduced by cloning of a degenerate synthetic V3 DNA fragment. These amino acids are predominant within the hypervariable regions on either side of the conserved CPGGRAF motif of the B subtype consensus sequence. To identify V3 sequences which were highly reactive with different HIV-1 sera, a panel of 300 GST-V3 clones were screened with a pool of 40 HIV-1-positive sera. These 40 sera were from asymptomatic patients and were not identical to the sera used for the screening of V3-specific antibody over time. In this preliminary screening 10 highly reactive GST–V3 fusion proteins were identified (Fig. 1, SYN) which were used to detect the loss of V3-specific antibody.

When the 90 sera taken at an early stage of infection were tested against the SYN V3 domains, sera of 47 patients reacted with all 10 GST–V3 fusion proteins. When the sera were grouped according to the number of GST–V3 proteins recognized (Fig. 2) a mean reactivity of 95% was calculated. The same result was obtained based upon the reactivity of each single GST–V3 fusion protein (88–99%, Fig. 1).

In Fig. 3 the anti-V3 reactivities of early and late serum samples of 15 of the 90 patients are shown. A clear reduction (>75%) of the anti-V3 reactivity directed against a single V3 domain was seen over time. In the late sera of six patients (A4, A5, B11, C10, C11, D9), only a weak reaction was found while nine patients completely lost V3-specific antibodies (A1, B9, B4, D4, E1, E10, F4, F11, H4).

In addition to the SYN GST–V3 fusion proteins another 11 GST–V3 fusion proteins (Fig. 1, PAT) were applied using various V3 loop sequences obtained from wild-type env gene sequences identified in different patients (Schreiber et al., 1994). When these 11 GST–V3 fusion proteins were tested with the 90 paired sera and the number of reactive sera was plotted against the number of V3 domains recognized, a Gaussian distribution was seen (Fig. 2). Most of the patients’ sera recognized between six and eight different GST–V3 fusion proteins of the PAT V3 domains. At the Gaussian distribution maximum a mean reactivity of 64% was found and was
identical to the mean reactivity based upon data of each single GST–V3 (54–74%, Fig. 1).

In Fig. 4 (a–f) (left side) the results of the antibody screening against the PAT V3 domains are shown. In six of the 90 patients (A7, B4, D5, D8, D9, and H2) a significant decrease (66%) of reactivity directed against a single V3 domain of one of the 11 GST–V3 fusion proteins was detected within 5–7 years. In all six patients antibody reactivities were also monitored at annual intervals (Fig. 4a–f, right side). Thus a progressive decrease of antibody reactivity was confirmed for patients D5, D8, D9 and H2. In patient A7 a rapid antibody loss against the C1-01 V3 domain was seen within 1 year, while in patient B4 a discontinuous decrease was observed reaching the cutoff level in 1992. The loss in patient D9 was detected with both groups of GST–V3 antigens. This patient lost antibodies not only against the PAT C2-03 V3 loop but also against five V3 domains of the SYN group (Syn01, Syn05, Syn06, Syn07, Syn09). In all six patients antibody titres to gp120/gp160 as measured by Western blot (Bio-Rad) and the total number of B cells as measured by FACS analysis (CD19+ cells) were stable over time (data not shown).

During the period when the loss of antibody was found, 15 of the 20 patients progressed to AIDS as documented by clinical symptoms and decreasing CD4+ T cell counts (A4, A5, A7, B4, B8, B9, B11, C10, D4, D5, D8, D9, E10, F11, H2). CD4+ T cell counts remained stable over time in patients F4, E1 and H4, reaching 500, 770 and 510 CD4+ T cells/μL, respectively, at the time when V3-specific antibody was completely lost. Two patients had a decline of CD4+ T cells from 870 to 330 cells/μL during an interval of 6 years. For the six patients who were identified by screening PAT V3 domains, the drop of CD4+ T cells from 200 below 50/μL and progressed to AIDS is indicated by the grey areas in Fig. 4 (a–f) (right side).

To summarize: 20 patients who had lost antibodies against a single V3 domain, but showed stable reactivity against closely related V3 domains could be identified in the group of 90 patients. Fifteen patients were identified by screening SYN V3 domains with a mean seroincidence of 95% and five other patients were identified using PAT V3 domains with a mean seroincidence of 64%. From these results we conclude that the probability of detecting the loss of a V3-specific antibody depends mainly on the number of V3 domains applied and on the frequency with which an individual V3 domain is recognized by serum antibody.

Loss of autologous V3-specific antibodies and viral escape

To analyse the V3-specific antibody response directed against autologous V3 domains, we first characterized the
Early

Loss of heterologous antibody against the SYN V3 domains. The serum response of 90 patients was monitored by V3-ELISA over more than 4 years. Sera of 34 patients showed a constant response, 23 patients showed an increasing response and 19 patients showed a decline in V3-specific antibody reactivity (40–75% reduction in V3-ELISA). The loss of a V3-specific antibody to a single V3 domain was detected in 15 patients (75–100% reduction in V3-ELISA). Black bars, V3 reactivity at the early stage. Grey bars, V3 reactivity at the late stage. Loss of antibody: Syn01, D4; Syn02, E1; Syn03, E10; Syn05, H4; Syn06, A11, B9, B11, C10, C11, D4, F11; Syn07, A4, F4; Syn08, A5; and Syn09, B8. Time interval: A4, 4 years; A5, 7 years; A11, 6 years; B8, 10 years; B9, 6 years; B11, 7 years; C10, 6 years; C11, 6 years; D4, 5 years; D9, 6 years; E1, 5 years; E10, 7 years; F4, 8 years; F11, 5 years; H4, 4 years. Dotted lines indicate the cutoff level calculated from a pool of HIV-1-negative sera and degrees of positivity as indicated in Table I.

intra-patient iCFV and non-iCFV V3-variants (Table 1). The iCFV and non-iCFV variants were analysed from serum samples of both patients in 1992. The genomic viral RNA was amplified by RT–PCR. The amplified DNA was cloned and for each experiment 20 clones were sequenced to characterize the frequency of the variants found. In patient C, two variants C2-07 and C2-13 were identified as the predominant cell-free species. F2-06 was the predominant V3 variant in patient F. When the serum samples taken in 1992 were used for the identification of the iCFV, it turned out that the two variants were not identical to the majority of non-iCFV virions. For patient C variant C1-01 and for patient F variant F1-01 was isolated as the iCFV variant. Thus both iCFV variants must have been a minor fraction (< 5%) among the cell-free viral population in the serum.

To study the autologous antibody response the sequences of iCFV and non-iCFV were expressed as GST–V3 fusion proteins and used for the detection of V3-specific antibodies by ELISA. The antibody response to the iCFV and non-iCFV V3 domains of patients C and F was monitored retrospectively at annual intervals. For patients C and F the first serum was available in 1986 and 1988, respectively. As shown in Fig. 5 the antibody reactivity against the major non-iCFV V3-domains C2-07, C2-13 and F2-06 did not change significantly in the interval of 6 (patient C) and 5 (patient F) years and were comparable to the reactivities against the iCFV V3-domain in the asymptomatic phase of the disease. In contrast, in the serum samples from 1992 used for iCFV isolation autologous antibody directed against the iCFV V3 domains was no longer detectable (Fig. 5, arrow). The autologous antibody reactions against the non-iCFV and iCFV V3 domain could be confirmed under stringent ELISA conditions (1–6 M-urea, room temperature) indicating a high affinity/avidity of the V3-specific antibodies. Also, anti-iCFV V3 antibody could not be detected after TCA or urea treatment of serum samples. At the same time anti-HIV-1 titres increased or remained stable and the loss of polyclonal immunoglobulins was not detectable by Western blot (Fig. 6). In serum samples taken before 1992 (stored at −70 °C), and positive for iCFV V3-specific antibody, no iCFV could be isolated.

At the time of first serum sampling both patients had normal CD4+ T cell counts and were asymptomatic without clinical symptoms (patient C, 1986, CD4+ count 600/µl; patient F, 1988, CD4+ count 800/µl). In both patients a concomitant decline of CD4+ T cells from 200 to below 50 cells/µl (grey areas in Fig. 5) could be observed. This time interval of CD4+ T cell decline parallels the period when the iCFV V3-specific antibody was lost followed by the rapid development of full blown AIDS in both cases.

Taken together, the loss of V3-specific antibody was also
Fig. 4. Loss of heterologous antibody against the PAT V3 domains. Left-hand side, the serum response of 90 patients was monitored by V3-ELISA over more than 4 years. Sera of 28 patients showed constant reactivities, 61 sera revealed increasing antibody reactivities. Only one was not reactive. In the case of six patients shown (a-f) the loss of a type-specific antibody reactivity was detected versus a single V3 domain (black boxes). Grey boxes indicate GST-V3 fusion proteins with constant antibody reactivities and the white boxes denote V3 domains with increasing antibody reactivities. Representative of eight...
experiments. On the right, for each patient the V3-specific antibody kinetics for three different V3 domains were monitored at annual intervals. Black dots indicate declining, grey dots, constant, and white dots increasing anti-V3 antibody reactivities. Dotted lines indicate the cutoff level calculated from a pool of HIV-1-negative sera and degrees of positivity as indicated in Table 1. Representative of eight experiments. Grey areas indicate the time interval, in which CD4+ T cell counts decreased from 200 to < 50 cells/µl and patients progressed to AIDS.
Table 1. Frequency of iCFV and non-iCFV variants in patient C and F analysed in 1992*

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* Percentage of the cell-free V3 variants monitored by sequencing of 20 independent clones; 20 of 20 = 100%.

† The autologous antibody response to GST-V3 fusion proteins as measured by ELISA, see Fig. 5 (—, < cutoff; +, < 0.33; ++, < 0.66; +++ + , > 0.66).

confirmed by the study of autologous immune responses in both patients. Moreover, the loss of a V3-specific antibody was linked to the release of the corresponding virus variant.

Discussion

Escape of HIV-1 from the humoral immune response is well documented and is thought to result from extensive viral replication with the concomitant development of mutants that are no longer recognized by the immune system (McKeating et al., 1989; Nara et al., 1990). In addition to this phenomenon, we found a continuous decrease of the V3-specific antibody over time followed by the escape of the corresponding virus variant, the iCFV. The loss of autologous antibody was shown in two AIDS patients (C and F) indicating the progressive and V3-specific impairment of the humoral immune response. The
antibody loss was confirmed in a heterologous screening experiment. In this experiment a further 20 patients were identified also showing an impaired response against certain V3 domains.

A similar immunological impairment during disease progression was shown by Pincus et al. (1994) who analysed the antibody response to HIV-1 envelope protein in IIIB-infected laboratory workers over time. They found that anti-Env and anti-V3 antibody reactivity was linked to clinical progression. The laboratory worker who had developed AIDS showed the lowest antibody levels. In a follow-up study Lu et al. (1993) reported on HIV-1-infected patients with rapid loss of CD4+ T cells showing significant reduction of neutralizing antibody titres against autologous virus variants. Moreover, low levels of antibodies directed against the p24 or p17 proteins have been shown to be correlated with progression to AIDS (Allain et al., 1987; Lange et al., 1986) and were not due to formation of immune complexes (Fenouillet et al., 1993). Taken together, during progression to AIDS, neutralizing antibodies or antibodies directed against gp120 and V3 decrease in a selective but unknown way (Page et al., 1992; Cavacini et al., 1993; Fenouillet et al., 1995). Besides this impairment of the anti-HIV immune response, an impairment of the antibody response against parasite antigens was also observed in patients coinfected with HIV-1 and malarial or leishmanial parasites. A loss of parasite-specific antibodies was observed only in HIV-1-infected individuals (Gradoni et al., 1993; Wabwire-Mangen et al., 1989). A number of different mechanisms might account for the impaired immune response. Selective depletion of memory CD4+ T cells was demonstrated in HIV-1-infected patients (Schnittman et al., 1990). As HIV-1 replicates preferentially in CD4+ T cells of the Th2-type (Maggi et al., 1994) this would affect the subset of antigen-specific CD4+ T cells resulting in a decline of T helper cells responsible for B cell proliferation. Furthermore, the impairment of the antibody response during the course of HIV-1 infection could also be due to HIV-mediated defects in antigen presentation (Macatonia et al., 1990). When the loss of a V3-specific antibody was monitored in each of our patients, reappearance of a lost V3-specificity was not observed in spite of the presence of the corresponding pathogen, the icFV. This immunological tolerance phenomenon may be due to the clonal deletion or active suppression of the V3-specific B lymphocytes. Pulendran et al. (1995) carried out experiments where mice were treated with high doses of 4-hydroxy-3-nitrophenyl acetic acid coupled to a soluble protein carrier. They showed that soluble antigen given after challenge immunization was able to impede both the formation of antigen-specific germinal centres and the affinity maturation of antigen-specific cells. But at the same time it enhanced immunoglobulin secretion in cells without regard to the antigen specificity. This phenomenon is also seen in the late stage of HIV-1 infection. Pulendran et al. (1995) discussed their results in relation to infections by Plasmodium falciparum, which causes malaria, which can provide a quasi-species-like milieu of epitopes, expressed as tandemly repeated sequences, that cause excessive proliferation of B cells and frustrate affinity maturation (Anders, 1986). In chronic HIV-1 infection the situation may be similar because many different virus variants shed large amounts of closely related envelope proteins into the blood of their host irrespective of the production of intact or imperfect viral particles (Layne et al., 1992). The continuously shifting and turnover of a high number of closely related antigens might be the main reason that the cellular memory fails. On the other hand, the loss of antibody could be due to the decline of CD4+ helper T cells, which are responsible for the proliferation of B cells. But as can be seen in our screening results in Fig. 4 (a-f), despite the loss of a V3-specific antibody and the decline of CD4+ T cells in all six AIDS patients, a simultaneous increase of new antibody reactivities was observed. Thus, even in the late stage of the disease, B cell clones are able to proliferate and can produce various V3-specific antibodies, in spite of low CD4+ T cell numbers. In patients C and F the autologous antibody response able to neutralize the majority of the cell-free virions (C2-7, C2-13, F2-06) was stable over time. Shortly before death, when CD4+ T cell levels were below 10/μl, only a weak reduction of the antibody reactivity to non-icFV V3s was observed in both patients. Thus an effective antibody response was maintained despite the progressive loss of T helper cell function in both patients.

Our findings of the impairment of the humoral immune response in HIV-1 disease are focused on cell-free virions and the neutralizing activity of antibodies. In addition to antibodies, CTLs are highly efficient in clearing virus (Pircher et al., 1990). In parallel to our observations, the memory cell fraction of these antiviral effector cells (CTLm) is also lost in AIDS patients (Rinaldo et al., 1995). In this study, a complete loss of HIV-1 specific CTLm was observed in patients who progressed to AIDS in contrast to long-term non-progressors.

Our findings on V3-specific antibody loss raised the question of whether the loss of antibody could be confirmed by a randomly designed experiment where sera of patients were screened for reactivity against various V3 domains. To this end, sera were tested against V3 sequences derived from patients and V3 sequences selected from a GST-V3 library. This library represents variants of the HIV-1 B subtype consensus sequence. The B subtype has been studied most intensively and is predominantly found in HIV-1-infected individuals from North and South America, Europe and Asia. It is not surprising therefore, that variants of the B subtype V3 sequence showed a mean reactivity of 95% in our ELISA. The mean reactivity of V3 domains from the patients (PAT, 64%) was similar to those identified by LaRosa et al. (1990) who also tested V3 sequences with 86 sera from HIV-1 patients.

Considering that the V3 loop is the most variable domain in the gp120 protein and the presence of millions of HIV-1 variants in one patient is documented (Wain Hobson, 1995) it seems to be very unlikely that we would be able to identify a
corresponding autologous iCFV by our heterologous screening. Such a corresponding variant would be detected only by chance. In contrast to this, the proportion of patients (20/90; 22%) identified by our test seems to be quite high. Thus the loss of V3-specific antibodies seems to be a rather frequent event in progressive HIV-1 disease. Moreover, the random design and the use of different sets of V3 domains is also suggestive of frequent antibody loss in AIDS patients. When the loss of antibody is documented by using a certain number (n) of V3 domains and more patients are identified using more V3 domains (n + 1) one may conclude that the loss of V3-specific antibody can be found in almost all 90 patients and might be a general event in HIV-1 disease.

If 75% (15 of the 20 patients) who lost V3-specific antibodies progressed to AIDS and only 55% were AIDS progressors among the other 70 patients, a shift to more AIDS patients in the group with V3 antibody loss was observed. Although the design of the heterologous V3 antibody screening allows the detection of antibody loss, no statistically significant correlations between antibody loss and CD4+ cell decline can be drawn. Because the V3 antibody response is highly specific, the loss of V3 antibody can only be detected if the V3 domain fits completely, but this occurred by chance only. As Coombs et al. (1989) have shown that the isolation of neutralization-resistant escape variants is more likely in AIDS patients, future work is needed to also examine the quantity of V3-specific antibody loss against many more different V3 sequences, as a possible mechanism for viral escape in relation to disease progression.

In conclusion, an impaired response of the immune system during disease progression seems to be a result of the selective loss of both B cell memory (Pulendran et al., 1995) and CTLm (Rinaldo et al., 1995) clones. These memory defects might cause irreversible ‘holes’ within the armoury of antiviral effector cells. Subsequently, virus variability will result in the development of variants which by chance can break through these ‘holes’. This selective immune defect disturbs the sensitive balance in the struggle against HIV-1, allowing certain variants to escape from immune surveillance.

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