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A longitudinal study of the IgG antibody response to HIV-1 p17 *gag* protein in HIV-1$^+$ patients with haemophilia: titre and avidity

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SUMMARY

The IgG response to HIV-1 p17 *gag* protein was studied for up to 6 years in 12 HIV-1-infected patients with haemophilia, who had seroconverted between 1982 and 1985. To assess any prognostic value, p17 IgG titres were compared with p24 IgG titres, CD4 cell counts and p24 antigenaemia. p17 IgG avidity index was also examined. A strong similarity was found between the IgG titre to HIV-1 p17 and that to p24. In patients who developed AIDS the decline in p17 IgG titres could precede by several years the drop in CD4 cells to under 200 cells/μl; whereas some long-term asymptomatic patients (CDCII) had increasing p17 IgG titres and stable CD4 cell counts. Declining p17 and p24 IgG titres were not always associated with an increase in p24 antigenaemia. IgG titres were found to be better predictors of disease progression than CD4 cell counts or p24 antigenaemia. Patients who developed AIDS during the study were also characterized by a lower p17 IgG avidity than patients who remained asymptomatic . This result suggests that IgG avidity could have prognostic relevance and be of importance for host resistance to AIDS onset.

Keywords HIV-1 *gag* antibody response p24 antigenaemia avidity prognostic markers

INTRODUCTION

Infection with HIV-1, the etiological agent of AIDS [1], is characterized by a long asymptomatic phase. Initially HIV-1 induces a strong humoral immune response to the principal structural components of the retrovirus. Antibodies to proteins encoded by the genes *env* (gp120 and gp41), *gag* (p24 and p17) and *pol* (p66 and p31) can be detected following HIV-1 infection [2–6]. Antibody levels to *gag* proteins fall during the progression to AIDS, while little change in antibody levels to gp120 and gp41 occurs [5,7,8]. A decline in the antibody response to p24 has been considered to be of prognostic value [7–9], and an earlier indicator of disease onset than a change in CD4 cell counts [9]. Recently there has been an interest in monitoring immune responses to HIV-1 p17, and several groups have reported that p17 antibody levels are a better serological marker of disease progression than p24 antibody levels [10–13]. No analysis of antibody affinity or avidity to HIV-1 *gag* proteins has yet been published. Comparable studies on the antibody responses to HIV-1 gp41 [14] and to hepatitis B virus (HBV) [15] demonstrated that low avidity or affinity antibodies were associated with viral pathology.

To investigate the humoral response to HIV-1 p17 and assess its potential clinical value, we have studied the IgG response longitudinally in HIV-1$^+$ patients with haemophilia in relation to their clinical status over a period of 5–6 years. We have analysed quantitatively the IgG response to p17 and its relationship with p24 IgG levels, p24 antigenaemia and CD4 cell counts. Finally, the avidity of the p17-specific IgG response was compared with each patient's clinical course.

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PATIENTS AND METHODS

Patients

A cohort of 12 patients with haemophilia A and infected with HIV-1 was followed between 1986 and 1993. Sera collected at 3–6 month intervals during this period were aliquoted and stored at -70°C. Two additional patients (18I and 19S) were included in the study of the IgG avidity.

According to clinical status (Table 1), the patients could be divided into two groups; those who remain asymptomatic (ASY) or CDC II to date, and patients who developed AIDS (CD4$^+$ cell counts $< 200/\mu$l of blood) after 1 January 1986 [16]. This date was chosen as zero-time and subsequent time-points are given in months from this reference.

Recombinant proteins

HIV-1_{HXB2} recombinant p17 and p24 (rp17 and rp24) were supplied by the MRC AIDS Directed Programme (gift from Dr G. Reid, University of Glasgow, UK). rp17 and rp24 were expressed in a pGEX system as glutathione S transferase fusion

Table 1. Patients' clinical status

Patients	Age 1 January 1986	Last date HIV-1$^-$	First date HIV-1$^+$	Clinical status 31 January 1993	Zidovudine (AZT) treatment
01H	51	NA	January 84	CDC II	–
03D	19	August 82	September 83	CDC II	–
05B	33	NA	November 83	CDC II	–
06T	26	NA	November 84	CDC II	Since month 50
07F	46	August 84	September 84	CDC II	Since month 67
10G	37	January 85	October 85	CDC II	–
11G	21	June 82	July 82	CDC II	Since month 74
09L	28	NA	May 84	AIDS (70)*	Months 52–74
02Q	63	October 84	August 85	AIDS(27)*/death(63)*	–
04L	32	December 84	June 85	AIDS(24)*/death(46)*	Months 25–46
08B	29	NA	November 83	AIDS(29)*/death(59)*	–
17P	29	December 82	March 83	AIDS(17)*/death(65)*	–
18I	57	NA	September 83	AIDS(53)*/death(73)*	Months 59–73
19S	56	February 84	May 84	AIDS(22)*/death(34)*	–

* Onset of AIDS (CD4$^+$ cells $< 200/\mu l$) and date of death in months from 1 January 1986. All patients were classified as CDCII at entry (1 January 1986). NA, not available.

proteins in *Escherichia coli* and were purified in a single step chromatography [17].

ELISA for IgG to HIV-1 p17 and p24

High binding EIA/RIA microplates (Costar, High Wycombe, UK) were coated with 50 μl of rp17 or rp24 at 3 μg/ml in PBS pH 7·2 for 3 h at 22°C. Free sites were blocked with 200 μl of PBS-bovine serum albumin (BSA) 2·5% for 2 h at 37°C. The plates were washed with a standard procedure: six times with PBS-Tween 20 0·1% (PBS-Tw) using an automatic plate washer (Coulter, Luton, UK).

Human sera were diluted in PBS-Tw BSA 2·5% (from 1:100 and 0·5 log serial dilutions thereof) and 100 μl of each dilution were incubated in duplicate overnight at 4–8°C. Plates were washed and 100 μl of anti-human IgG peroxidase conjugate (ref. P214; Dako, High Wycombe, UK) at a dilution of 1:5000 were incubated for 1 h at 37°C. After washing, H_2O_2 and *o*-phenylenediamine were added as substrates for 15 min at 22°C. The enzymatic reaction was then stopped with 2·5 M H_2SO_4 and absorbance (A) was measured at 492 nm on a Titertek Multiscan (Flow Laboratories, UK). IgG titres were endpoint dilutions for $A_{492} = 0{\cdot}2$.

Sera from 10 patients with haemophilia who were HIV-1$^-$ were used as negative controls; these sera diluted 1:100 gave a mean $A_{492} = 0{\cdot}128$ ($\pm 0{\cdot}036$) and $A_{492} = 0{\cdot}115$ ($\pm 0{\cdot}024$) for p17 and p24 IgG assays, respectively.

Avidity assays for IgG to HIV-1 p17

Urea (denaturant) and KSCN (chaotropic ion) were employed for immune complex dissociation and estimating avidity of polyclonal antibodies [14,18,19].

First, the stability of rp17-solid phase (see ELISA for IgG) was tested in presence of 6–8 M urea and 2–6 M KSCN for 30 min at 22°C. Since rp17-solid phase was affected by KSCN 4–6 M (approximately 25% reduction in reactivity), urea was chosen for testing the IgG avidity.

Four replicates of each working dilution of the sera were incubated as described in the ELISA for IgG. After three washes, one duplicate received 100 μl of urea 8 M in PBS and 100 μl of PBS were added to the second duplicate for 15 min at 22°C. Urea was then removed by six washes with PBS-Tw and IgG bound to the solid phase were detected with the anti-human IgG peroxidase conjugate.

The avidity index (AI) was calculated as follows: $AI = Ax$ (mean A_{492} duplicate urea treated)/Ao (mean A_{492} duplicate PBS) $\times$ 100. To maximize the sensitivity and reproducibility of the assay, sera working dilutions were chosen to have $Ao = 1{\cdot}0 \pm 0{\cdot}1$.

HIV-1 p24 antigen assays

p24 antigen was titrated with or without immune complex dissociation (ICD). For ICD, 100 μl of sera were acidified with 100 μl of glycine-HCl 1·5 M pH 1·85 for 90 min at 35°C and then neutralized with 100 μl of Tris-HCl 1·5 M pH 9·0. Without ICD 100 μl of sera were diluted with 200 μl of neutral buffer (glycine-HCl 1·5 M:Tris-HCl 1·5 M, 1:1). Then, 200 μl of neutralized sera were titrated either with Coulter p24 antigen kit or with a sensitive fluorescent-ELISA for p24 we recently described for monitoring HIV-1 replication *in vitro* [20]. The fluorescent-ELISA was slightly modified for detection of p24 antigen in human sera, MoAb EH12E1 [21] was used as capture antibody, and biotinylated human IgG (from a pool of HIV-1$^+$ sera) was used as detection antibody, at 1·5 μg/ml. Antigen standard solutions (rp24) were prepared in PBS 33% fetal calf serum 0·5% Triton X-100. The other parts of the protocols remained identical to those described [20].

CD4 and CD8 cell counts

CD4$^+$ and CD8$^+$ T cell counts were assessed in whole blood by flow cytometry on FACScan (Becton Dickinson, UK), using fluorochrome-labelled MoAbs to CD4 (Leu-3a) and to CD8 (Leu-2a).

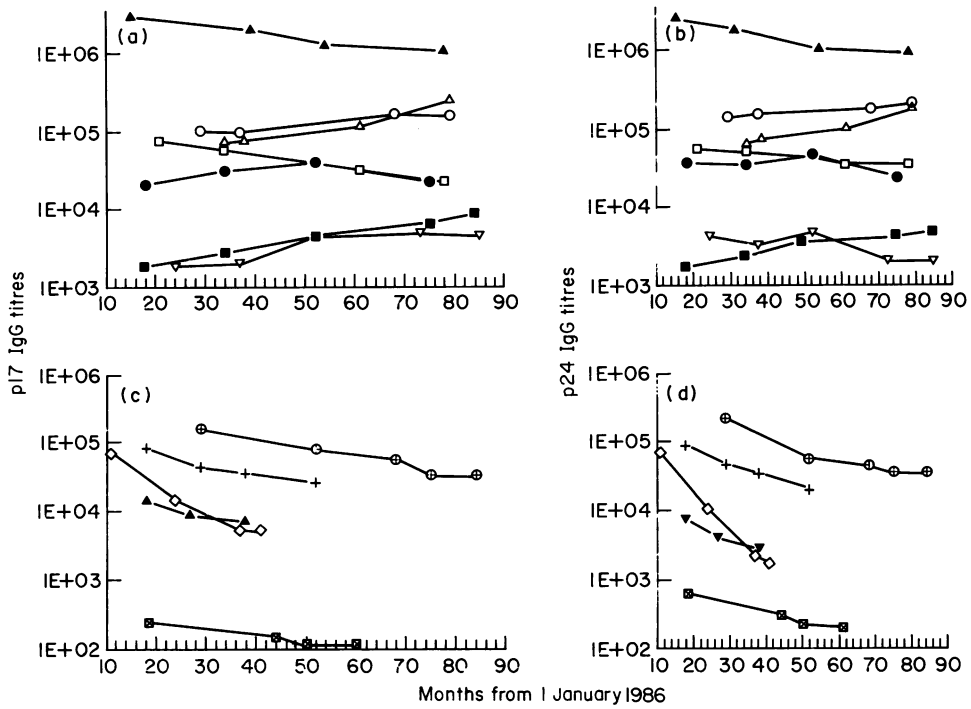

Fig. 1. Comparative longitudinal analysis of the IgG response to HIV-1 p17 and p24: p17 and p24 IgG titres for asymptomatic (a, b) and for AIDS patients (c, d). Patients 06T, 07F, 11G, 04L and 09L received Zidovudine (AZT) treatment (see Table 1). ○, 01H; □, 03D; △, 05B; ▽, 06T; ●, 07F; ■, 10G; ▲, 11G; ▼, 02Q; ◇, 04L; +, 08B; ○, 09L; □, 17P.

Table 2. Longitudinal analysis of p24 antigenaemia without and with immune complex dissociation (ICD)

Patients	01H	02Q	03D	04L	05B	06T
Months from 1 January 86	29-37-68-79	18-27-38	21-34-52-78-85	11-24-37-41	34-39-62-79	24-36-50-73-85
p24 (pg/ml)	00-00-00-00	00-00-00	00-00-00-00-00	00-00-00-00	00-00-00-00	00-26-227-30-67
p24 with ICD (pg/ml)	06-05-00-00	14-12-13	09-09-09-13-04	19-128-31-16	00-13-17-18	107-197-379-165-107
Patients	07F	08B	09L	10G	11G	17P
Months from 1 January 86	17-34-50-75	29-37-40-53	29-54-69-75-79	15-35-49-74-83	15-31-54-78	18-44-50-61
p24 (pg/ml)	00-00-07-00	102-500-113-125	00-00-13-65-00	00-00-00-00-00	00-00-00-00	02-61-06-19
p24 with ICD (pg/ml)	22-16-16-17	558-849-466-517	45-37-56-78-18	10-16-17-14-08	13-17-10-11	125-124-41-181

RESULTS

p17 IgG titres: longitudinal analysis and relationship to p24 IgG titres, p24 antigenaemia and CD4 cell counts

Titres of IgG antibodies to HIV-1 p17 were analysed longitudinally over a period of 3–6 years, depending on the clinical course of the patients (Table 1). An indirect ELISA was used to quantify the levels of specific IgG directed against p17 in each serum sample. Cross-sectional results showed that p17 IgG titres varied widely, from $2{\cdot}8 \times 10^6$ for patient 11G to 100 for patient 17P, and did not relate to clinical status (Fig. 1). As shown in Fig. 1, all patients who developed AIDS since 1986 had declining titres; whereas in the asymptomatic group two opposing patterns were observed: two patients (03D and 11G) had a constant decline in IgG titres, but increasing titres were observed for four patients (01H, 05B, 06T and 10G) over a 4–5 year period.

Comparison with p24 IgG titres (Fig. 1) revealed a quantitative similarity between the IgG responses to these two antigens. Significantly, patients with low p17 IgG titres also had low p24 IgG titres and *vice versa*. Furthermore, on a longitudinal basis, titres to p17 and p24 showed parallel evolution in all patients, with the exception of patient 06T, who had a steady increase in p17 IgG titres accompanied by a decline in p24 IgG titres between months 50 and 73.

The p24 antigen concentration with or without ICD was measured in each patient's serum (Table 2). ICD treatment increased dramatically the detectability of p24 antigen in sera. In a total of 96 sera analysed, 73% which were initially negative for p24 antigen became positive after ICD ($\geqslant$10 pg/ml). The relationship between IgG titres and p24 antigenaemia was examined (Fig.1 and Table 1); patients 04L, 08B and 17P who had a steady decline in IgG titres also had high p24 antigenaemia. Other patients, however (02Q, 09L and 03D) with declining titres did not have a marked rise in p24 antigen concentration.

The longitudinal relationship between IgG titres and CD4 cell counts was also examined, and the results for four patients are represented in Fig. 2. Patients 01H and 10G had a sustained increase in IgG titres and very stable CD4 cell counts over the period of study. Patients who developed AIDS (04L and 09L) by

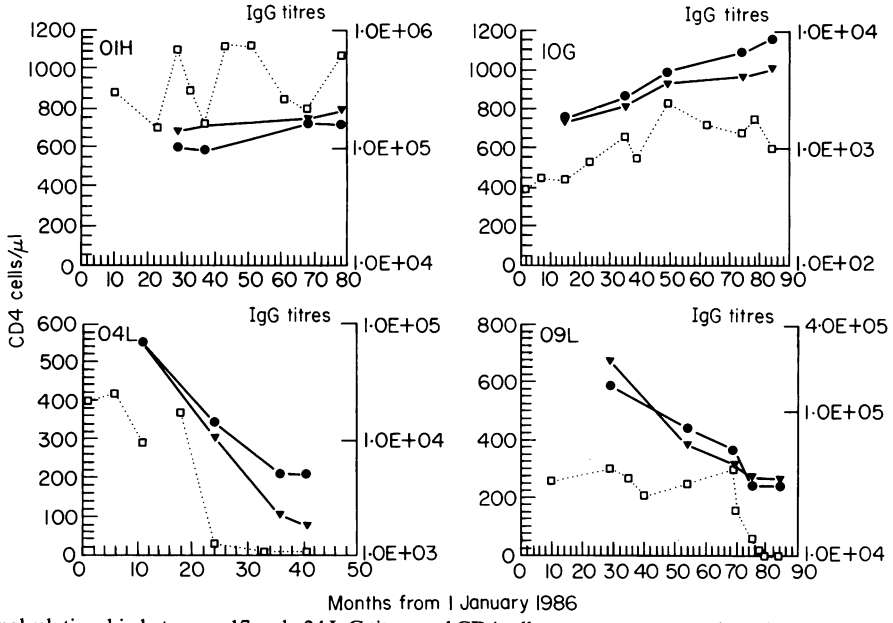

Fig. 2. Longitudinal relationship between p17 and p24 IgG titres and CD4 cell counts: asymptomatic patients (01H and 10G) and AIDS patients (04L and 09L).□, $CD4^+$ cells; ●, p17 IgG; ▼, p24 IgG.

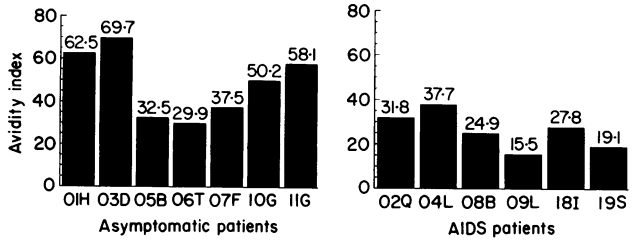

Fig. 3. Avidity index of the IgG response to HIV-1 p17. Two-sample *t*-test showed a significant difference in avidity index between the asymptomatic and AIDS patients (log transformed data, $P < 0{\cdot}01$). Sera of patient 17P had too low p17 IgG titres to be tested for avidity.

definition had low CD4 cell counts declining to under 200 cells/μl. However, as shown for patient 09L, the decline in IgG titres preceded by at least 40 months the fall in CD4 cells to below 200/μl (Fig. 2). Patients 03D and 11G (both asymptomatic), who had a steady decline in p17 and p24 IgG titres, maintained stable CD4 cell counts around 400–500 cells/μl.

Six patients received Zidovudine (AZT) treatment (Table 1), but no measurable effect was observed on p17 and p24 IgG titres (Fig. 1), despite a temporary reduction in p24 antigenaemia in some cases (patients 04L and 06T, Table 2).

p17 IgG avidity

Urea (a denaturant), which causes dissociation of immune complexes, was used to test the avidity of p17 IgG in each serum. Treatment with urea at high molarity (8 M) did not affect rp17 solid phase and was found to be optimum to differentiate low from high avidity IgG. To compare patients and serum samples of different dates, the assay was optimized with serum dilutions giving a similar signal level ($A_{492} \approx 1{\cdot}0$) in the ELISA for IgG.

An AI over 40 was only observed in the asymptomatic group, whereas the AIDS group was characterized with several AI $\leqslant 25$, and patients with AI between 30 and 40 were found in both groups (Fig. 3). Comparison by a two-sample *t*-test showed a significant difference between the two groups ($P < 0{\cdot}01$). Using the same urea method, avidities were also tested longitudinally, but no within-patient difference was observed (two-way analysis of variance, $P > 0{\cdot}1$), with the exception of patient 03D who had a drop in AI from 81 to 59 over 5 years. The AI value given for patient 03D (Fig. 3) is the median value at month 52.

DISCUSSION

The antibody response to HIV-1 *gag* p17 protein has been studied using cross-sectional sera [10,11,13]; however, a single time-point measurement per patient cannot adequately represent the humoral response to p17. A longitudinal approach provides a more powerful analysis, as recently shown for the antibody response to HIV-1 p24 [9]. We therefore considered it pertinent to study a patient cohort thoroughly to understand the natural history of the IgG response to HIV-1 p17. The patients with haemophilia chosen have been followed clinically at regular intervals since 1986. To characterize the IgG response to HIV-1 p17 gag protein, several parameters were analysed: titre and its relationship with p24 IgG titre, p24 antigenaemia and CD4 cell counts, and avidity.

At any single time-point the magnitude of the IgG response to p17 varied widely between patients, and inter-patient titres were not related to the clinical status; consequently a cross-sectional analysis was not informative. Analysis by Western blot showed the same trend; sera with strong p17 and p24 reactivities on Western blot had high IgG titres in ELISA, and sera with low ELISA titres were weakly positive or negative on Western blot (data not shown). Longitudinal observations showed no dichotomy between the IgG response to p17 and to p24, in contrast to data described by previous authors [10,11,13]. However, those authors did not titrate the p17 IgG response longitudinally. In this study all patients who developed AIDS were characterized by declining p17 and p24 IgG titres. When compared with CD4 cell counts, the decline in IgG titres could precede by at least 3–4

years the final decline in CD4 cells to under 200/μl, in agreement with results on p24 IgG titres [9]. For the asymptomatic patients (CDCII), stable or increasing p17 and p24 IgG titres were always associated with stable or increasing CD4 cell counts. Other clinically asymptomatic patients like 03D and 11G, who had declining IgG titres but low stable CD4 cell counts (400–500 cells/μl) could be in a phase proximate to the fall in CD4 cell counts, defining the onset of AIDS. These two patients had high CD8 cells counts (>1000 CD8/μl, data not given); previous studies have shown that some CD8 T lymphocytes can suppress both cell-mediated and humoral responses to HIV-1 [22]. Declining IgG titres can also result from early functional impairment in CD4 T helper cells [23]; HIV-1 infection is associated in some patients with a loss in immune responses to HIV-1 antigens [24] and to other viral antigens such as cytomegalovirus (CMV) [25]. Longitudinal measurement of total serum IgG concentration showed no correlation with increase or decrease in p17- and p24-specific IgG titres (unpublished data). Since IgG titres could directly reflect the functional status of CD4 cells, p17 and p24 IgG levels are better predictors of disease progression than CD4 cell counts. However, this requires that IgG titres and not only reactivity should be measured longitudinally for each patient. Such studies are essential for the rational planning and monitoring of immuno- or anti-viral therapies. In our studies, AZT treatment had no measurable effect on p17 and p24 IgG titres, and therefore is unlikely to have restored CD4 cell function.

p24 antigenaemia was estimated on the same patients' samples; p24 antigen appeared to be mainly in the form of immune complexes, since ICD treatment dramatically increased the level of p24 antigen detected [26,27]. However, as stated earlier [8], we found that p24 antigenaemia alone did not reflect the clinical status of the patients because patients with declining IgG titres and low CD4 did not necessarily have high p24 antigenaemia and *vice versa*. Therefore p24 antigenaemia was only informative when considered in the context of IgG titres and CD4 cell counts.

Overall AIDS patients had a lower IgG AI than asymptomatic patients. Two hypotheses could explain this result: patients who developed AIDS did not raise high avidity IgG to HIV-1 p17 following seroconversion; this could be due to a failure in affinity maturation of the antibody response to HIV-1. Low avidity antibodies would be less efficient in controlling the virus, thereby making such patients more vulnerable to disease development than those with higher avidity antibodies, as seen in hepatitis B infection [15].

Alternatively, a low IgG AI could result from a gradual loss in high avidity IgG to p17 with time after HIV-1 infection, as suggested for the IgG response to HIV-1 gp41 [14]. This is exemplified by patient 03D, who remains asymptomatic but has declining IgG titres and a fall in the AI over a 5-year period. The p17 IgG response was mainly restricted to subclasses 1 and 4, and IgG1 had higher avidity than IgG4 [15]; furthermore, IgG1 titres fell more dramatically than IgG4 titres (data not shown). This suggests that patients who developed AIDS had already lost proportionally more IgG1 [28] and higher avidity antibodies before 1986, as their IgG AI remained stable after that time. The IgG1 subclass may be more Th2 cell-dependent than other subclasses requiring IL-4 and IL-5 production [29,30], which may be deficient in HIV-1-infected individuals who develop AIDS [31].

Further work involving detailed measurement of mean affinity constants of the IgG response using double radioisotope methods described elsewhere [15,32] or a Bio-sensor equipment is essential to confirm the results found with the urea method. If similar results are obtained, the affinity of the IgG to HIV-1 will be a relevant early prognostic marker and would elucidate the role of the antibody response in controlling virus spread and pathogenesis, as observed for other viral infections [15,33]. However, as HIV-1 has a high mutation rate, a caveat should be made on the use of recombinant proteins or synthetic peptides for estimating IgG avidity/affinity. To complete the study of the IgG response to HIV-1 p17, the p17 *gag* gene of each patient's viral isolate should be sequenced to assess the influence of sequence variations on antibody affinity or avidity measurements.

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REFERENCES

1 Barre Sinoussi F, Chermann JC, Rey F *et al.* Isolation of lymphotropic retrovirus from a patient at risk for acquired immunodeficiency syndrome (AIDS). Science 1983; **220**:868–71.

2 Schupbach J, Haller O, Vogt M *et al.* Antibodies to HTLV-III in Swiss patients with AIDS and pre-AIDS and in groups at risk for AIDS. N Eng J Med 1985; **312**:265–70.

3 Barin F, McLane MF, Allan JS *et al.* Virus envelope protein of HTLV-III represents major target for antibodies in AIDS patients. Science 1985; **228**:1094–6.

4 Chou MJ, Lee TH, Hatzakis A *et al.* Antibody responses in early human immunodeficiency virus type I infection in haemophiliacs. J Infect Dis 1988; **157**:805–11.

5 Allain JP, Laurian Y, Einstein MH *et al.* Monitoring of specific antibodies to human immunodeficiency virus structural proteins: clinical significance. Blood 1991; **77**:1118–23.

6 McRae B, Lange JAM, Ascher MS *et al.* Immune response to HIV p24 core protein during the early phases of human immunodeficiency virus infection. AIDS Res Hum Retroviruses 1991; **7**:637–43.

7 Allain JP, Laurian Y, Deborah AP *et al.* Long-term evaluation of HIV antigen and antibodies to p24 and gp41 in patients with hemophilia. N Eng J Med 1987; **317**:114–21.

8 Cheingsong-Popov R, Panagiotidi C, Bowcock S *et al.* Relation between humoral responses to HIV gag and env proteins at seroconversion and clinical outcome of HIV infection. BMJ 1991; **302**:23–26.

9 Fenouillet E, Blanes N, Coutellier A *et al.* Monitoring of antibodies against human immunodeficiency virus type I p25 core protein as prognostic marker. J Infect Dis 1992; **166**:611–6.

10 Lange JMA, De Wolf F, Krone WJA *et al.* Decline of antibody reactivity to outer viral core protein p17 is an earlier serological marker of disease progression in human immunodeficiency virus infection than p24 decline. AIDS 1987; **1**:155–9.

11 Mehta SU, Rupprecht KR, Hunt JC *et al.* Prevalence of antibodies to the core protein p17, a serological marker during HIV-1 infection. AIDS Res Hum Retroviruses 1990; **6**:443–54.

12 Jiang JD, Chu FN, Naylor PH *et al.* Specific antibody responses to synthetic peptides of HIV-1 p17 correlate with different stages of HIV-1 infection. J AIDS 1992; **5**:382–90.

13 Baur A, Vornhagen R, Korn K *et al.* Viral culture and p24 antigenaemia of human immunodeficiency virus (HIV) infected individuals correlated with antibody profiles determined with recombinant polypeptides of all HIV-1 open-reading frames. J Infect Dis 1992; **165**:419–26.

14 Suni J, Huhtala ML, Korkolainen M *et al.* Avidity of HIV specific IgG-class antibodies. VIIIth International Conference on AIDS, Amsterdam 19–24 July 1992. Amsterdam: Congrex Holland B.V., Abstract PoA 2167.

15 Wen YM, Duan SC, Howard CR *et al.* The affinity of anti-HBc antibodies in acute and chronic hepatitis B infection. Clin Exp Immunol 1990; **79**:83–6.

16 CDC. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR 1992; **41**:1–19.

17 Smith DB, Johnson KS. Single purification of polypeptides expressed in *E. coli* as fusion glutathione-S transferase. Gene 1988; **67**:31–40.

18 Thomas HIJ, Morgan-Capner P. Rubella specific IgG1 avidity: a comparison of methods. J Virol Methods 1991; **31**:219–28.

19 Whitney C, Ant J, Moncla B *et al.* Serum immunoglobulin G antibody to *Porphyromonas gingivalis* in rapidly progressive periodontitis: titer, avidity, subclass distribution. Infect Immunol 1992; **60**:2194–200.

20 Chargelegue D, O'Toole CM. Development of a sensitive ELISA for HIV-1 p24 antigen using a fluorogenic substrate for monitoring HIV-1 replication *in vitro*. J Virol Methods 1992; **38**:323–32.

21 Ferns RB, Partridge JC, Spence RP *et al.* Epitope location of 13 anti-gag HIV-1 monoclonal antibodies using oligopeptides and their cross-reactivity with HIV-2. AIDS 1989; **3**:829–34.

22 Joly P, Guillon JM, Mayaud C *et al.* Cell mediated suppression of HIV specific T lymphocytes. J Immunol 1989; **143**:2193–201.

23 Shearer GM, Clerici M. Early T-helper cell defects in HIV infection. AIDS 1991; **5**:245–53.

24 O'Toole CM, Lowdell MW, Chargelegue D *et al.* Decline in CTL and antibody responses to HIV-1 p17 and p24 antigens in HIV-1 infected hemophiliacs irrespective of disease progression. A 5-year follow-up study. AIDS Res Hum Retroviruses 1992; **8**:1361–8.

25 Converse PG, Fehninger TE, Ehrnst A *et al.* Immune responses to fractionated cytomegalovirus (CMV) antigens after HIV infection. Loss of cellular and humoral reactivity to antigens recognized by HIV^-, CMV^+ individuals. Clin Exp Immunol 1990; **82**:559–66.

26 Mathiesen T, Sundqvist VA, Albert J *et al.* Acid hydrolysis of serum samples to increase detection of HIV antigen. J Virol Methods 1988; **22**:143–8.

27 Bollinger RC, Kline RL, Francis HL *et al.* Acid dissociation increases the sensitivity of p24 antigen detection for the evaluation of antiviral therapy and disease progression in asymptomatic human immunodeficiency virus-infected person. J Infect Dis 1992; **165**:913–6.

28 Broliden PA, Morfeldt-Manson L, Rosen J *et al.* Fine specificity of IgG subclass response to group antigens in HIV-1 infected patients. Clin Exp Immunol 1989; **76**:216–21.

29 Vitetta ES, Fernandez-Botran R, Myers CD *et al.* Cellular interactions in the humoral immune response. Adv Immunol 1989; **45**:1–106.

30 Del Petre GF, De Carli M, Mastromauro C *et al.* Purified derivative of *Mycobacterium tuberculosis* and excretory-secretory antigen(s) of *Toxocara canis* expand *in vitro* human T cells with stable and opposite (Type 1 T helper or Type 2 T helper) profile of cytokine production. J Clin Invest 1991; **88**:346–50.

31 Re MC, Zauli G, Furlini G *et al.* Progressive and selective impairment of IL-3 and IL-4 production by peripheral blood $CD4^+$ T-lymphocytes during the course of HIV-1 infection. Viral Immunol 1992; **5**:185–94.

32 Steward MW, Steensgaard J. In: Antibody affinity: thermodynamic aspects and biological significance. Boca Raton: CRC Press, 1983: 70–77.

33 Steward MW, Stanley CM, Dimarchi R *et al.* High-affinity antibody induced by immunization with a synthetic peptide is associated with protection of cattle against foot and mouth disease. Immunol 1991; **72**:99–103.