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Differential maturation of avidity of IgG antibodies to gp41, p24 and p17 following infection with HIV-1

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SUMMARY

We have evaluated solid-phase ELISA IgG antibody avidity studies as a means of identifying cases of recent HIV-1 infection. Although separate studies on the avidity of anti-gp41 and anti-p24 antibodies in seroconvertors have been reported, a comparison of the ability of patients to simultaneously mature their immune response to more than one HIV antigen immediately following seroconversion appears to be lacking. We have demonstrated a maturation in anti-gp41 avidity which reflects the time since seroconversion in all cases. In contrast, however, only some patients produced high-avidity anti-p24 or anti-p17 antibodies during the same time span. While the avidity of anti-gp41 antibodies remained high in cases of non-recent HIV infection, even in the face of advanced disease, we have confirmed the findings of others that the avidity of anti-p24 falls before the onset of ARC or AIDS. Therefore, whilst the avidity of anti-gp41 antibodies could reliably be of value in identifying cases of recent HIV infection, the avidity of anti-p24 or anti-p17 antibodies could not, but may be of prognostic value, even at an early stage. The time taken to reach maximum anti-p17, anti-p24 and anti-gp41 titres was variable, but anti-gp41 titres, like anti-gp41 avidity, remained high. In contrast, anti-p24 titres fell, even during the early follow-up period in some seroconvertors. Anti-p24 antibody avidity, however, appeared to be a better predictor of disease progression in 'remote' cases than anti-p24 titre. The avidity and titres of these antibodies are presented in relation to the clinical details, p24 antigen status, CD4 and CD8 counts where these are known.

Keywords anti-gp41 anti-p17 anti-p24 relative antibody avidity recent infection antibody titre

INTRODUCTION

Inappropriate timing of specimens can result in the indicators of early HIV infection, such as seroconversion, a rise in antibody titre, p24 antigenaemia, and specific IgM [1–3], being missed. Furthermore, although indicative of early infection, p24 antigenaemia may reappear in the later stages of the disease [4] and HIV-specific IgM detection can be problematical [5]. Whilst CD4 counts fall progressively in many HIV-infected cases [6,7], CD4 counts alone cannot be indicators of the duration of infection [8].

Functional affinity (avidity) is a measure of the strength of the bonds between antibodies and their corresponding antigens [9]. In the early stages of a primary immune response mainly low-avidity specific antibodies are produced. Both somatic hypermutation of V region germ-line genes and preferential selection of high-affinity B cells by antigen, especially declining concentrations of antigen, lead to an increase in the overall (average) avidity of specific antibodies over a period of months, and the response matures, usually after maximum antibody titres have been reached [10–14]. The eventual production of specific antibodies of high avidity is known to be beneficial [15].

This maturation of the antibody response has proved useful in serodiagnosis of viral infections for the timing of infection. Using sequential sera from cases of primary infection with a known date of onset of illness or seroconversion, an avidity maturation time course has been determined [14,16–18]. Such avidity studies are now of proven value in risk assessment in cases of rubella during pregnancy [14,19], where they can confirm recent infections in the face of equivocal IgM results and for some time after maximum IgG antibody titres have been reached.

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Analyses of sera from haemophiliacs have already shown the potential value of avidity tests in disease prognosis in HIV infection. Both the titre and avidity of anti-p17 and anti-p24 antibodies fell prior to the onset of AIDS, but the fall in avidity was a better predictor of disease progression than antibody titre [20–22].

Independent studies on antibody avidity in the early stages post-HIV seroconversion have shown avidity maturation of anti-gp41 antibodies [23] but very variable responses in the avidity of anti-p24 antibodies [24]. However, we are unaware of any studies on the simultaneous measurement of antibody avidity and titre to both env and gag proteins in cases early after seroconversion. Therefore, we have looked at the maturation of the humoral response (both antibody avidity and titre) to gp41, p24 and p17 in well defined cases of recent seroconversion and in other cross-sectional sera in an attempt to establish a method for identifying recent cases of HIV infection. In some cases insufficient serum was available for all tests. Results are related to clinical history and other pathology wherever possible.

MATERIALS AND METHODS

Sera

Sera were identified as HIV^+ if they were positive with (i) Behring Anti-HIV-1/-HIV-2 (Behring Hoescht UK Ltd, Hounslow, UK), and (ii) Abbott Recombinant HIV-1/HIV-2 third generation (Abbott, Maidenhead, UK), then with (iii) Serodia HIV passive particle agglutination test (Fujirebio Inc., Mast Group, Bootle, UK) and (iv) Liatek line immunoassay (Organon Teknika, Cambridge, UK).

$CD4^+$ and $CD8^+$ counts were carried out pre- and post-seroconversion for several patients.

Seroconvertor cases

Sequential sera (2–16 per patient) were collected from seven patients for up to 21 months following HIV seroconversion or recent illness compatable with HIV infection. Sera from patients 1–4 were collected at approximately monthly intervals. Single HIV^+ sera were collected from three other cases (patients 8–10) 1 month after an HIV-like illness and 14 and 18 days after HIV^- sera. All but two patients were homosexuals aged between 23 and 53 years. One patient was a female aged 39 years (patient 6), and one a 43-year-old heterosexual male (patient 10) with non-sexual close contact with an HIV^+ person [25]. Pre-seroconversion sera were available from six cases. A first serum equivocal for HIV antibodies in another case (patient 3) was followed by another 8 days later, which was positive. Three cases where no pre-seroconversion sera were available were deemed recent seroconvertors on clinical history, sexual contact and on the differential behaviour of their sera in the Behring peptide, Behring competitive and Abbott assays [26].

Patient 1 received Zidovudine for 10 days only, up to 1 day before the first serum assayed for antibody avidity. There was no record of anti-viral treatment in the other nine cases. All 10 seroconvertor patients were asymptomatic when their last sera were taken, and have remained asymptomatic. All were Caucasian and believed to have been infected in the UK.

Remote cases

Fifty-one sera from 37 cases aged 24–62 years, known to have been HIV^+ for longer than 12 months, including 14 cases of ARC or AIDS, were also tested. The avidity of both anti-p24 and anti-gp41 was studied in 24 of these sera. The avidity of anti-p24 alone was examined in a further 21 sera and the avidity of anti-gp41 alone examined in another six sera. All remote cases except one were caucasian. The one exception was an African women, resident in the UK for many years. Cases were classified as ARC or AIDS according to the criteria laid down by the Communicable Diseases Surveillance Centre, Colindale, UK. As CD4 counts fell below $0{\cdot}35 \times 10^9/l$, patients were offered AZT treatment.

Anti-gp41 titres were obtained from the serum dilution curves prepared for the avidity ELISA (see below) and expressed as the reciprocal of the serum dilution giving an absorbance reading at 492 nm (Abs_{492}) greater than the mean Abs_{492} of six HIV antibody-negative sera + 5 s.d.

Anti-gp41 avidity studies were carried out using an ELISA technique which expresses the avidity of antibodies under examination relative to the avidity of antibodies from cases of long-term immunity [16,17,19]. Behring Anti-HIV-1/-HIV-2 kits were adapted. Serum dilutions were prepared, in parallel, in serum diluent containing 50 mM diethylamine (DEA) (BDH, Poole, UK) and in serum diluent alone (used also for anti-gp41 titre—see above) in the wells of strips, incubated for 1 h at 37°C, and the wells were then washed six times with wash buffer. Solid-phase-fixed IgG was detected as described by the manufacturers. The reaction was stopped before the maximum Abs_{492} reached 2·0. Graphs of Abs_{492} against the reciprocal of the $\log_{10}$ of the serum dilution were plotted and the distance between the two curves measured (the DEA shift value [DSV]) [16]. Optimization of the DEA concentration was by chessboard titration with sera from cases early after seroconversion and from cases known to have been seropositive for longer than 1 year. Preliminary studies showed that <5% of the antigen was removed from the wells by the DEA treatment.

At least one acute serum and six sera from remote, asymptomatic cases of HIV infection were also included in each experiment and the mean DSV (x) and s.d. of the remote control sera calculated. A DSV index for each test serum was calculated [18] as:

$$\text{DSV index} = \frac{\text{DSV of test serum} - x}{1\,\text{s.d.}}$$

A DSV index >5 was taken to indicate mainly low-avidity-specific antibody, $\geqslant 3$ but $\leqslant 5$ was equivocal, and <3 indicated an insignificant amount of low-avidity-specific antibody (i.e. a mature response).

In order to establish that the enzyme-conjugated antiserum in the commercial kit was not detecting different antibodies from those detected in the other avidity tests, six acute and six remote sera were also tested on Behring kit plates with the goat anti-human IgG γ-chain peroxidase conjugate (horseradish peroxidase (HRP) anti-IgG) used in the anti-p24 studies (see below) instead of the conjugate supplied with the kit. No significant differences were observed.

Anti-p24 titres were taken as the reciprocal of the serum dilution which gave an Abs_{492} of 0·2 on the dilution curves (see below).

Anti-p24 avidity

The method of Hedman and colleagues [27] was adapted. Nunc Polysorp microtitre plates were coated with recombinant p24-GST (*Escherichia coli*) fusion protein at 2 μg/ml (ADP 634, MRC

Table 1. Avidity of anti-gp41, anti-p24 and anti-p17 antibodies in cross-sectional sera

Patient	Time (months) of sera post-seroconversion or illness	Avidity of specific antibodies in last serum taken		
		gp41	p24	p17
5	0·5, 1·5	Low	Low	Low
6	11*, 12	High‡	High‡	ND
7	1·5, 2, 5	Equivocal	Increasing	Low
8	1†	Low	Low	ND
9	18 days	Low	ND	ND
10	14 days	Low	ND	ND

* Sera taken at time 0 were characteristic of early seroconversion sera by their differential behaviour in tests of Watkins *et al.* [26].

† Serum taken 1 month after an HIV-like illness; also designated 'early' by tests of Watkins *et al.* [26].

‡ Antibody avidity was also high at 11 months.

ND, Test not done.

AIDS programme) in carbonate/bicarbonate coating buffer overnight at 4°C. Free solid-phase sites were blocked with 20% normal goat serum in PBS–Tween (NGS/PBS–T) for 1 h at room temperature. For each serum, two sets of dilutions were prepared in serum diluent (NGS/PBS–T) in the wells of the plates. Low- and high-avidity antibody controls, as above, were included. Following incubation at 37°C for 1 h, one dilution series was soaked at room temperature in serum diluent and the other soaked in diluent containing 100 mM DEA for 15 min. After washing six times, a standard ELISA was performed using a $1:10^4$ dilution of HRP anti-IgG (Tago, Inc., TCS Biologicals, Botolph Claydon, UK; code no. 2390) and orthophenylenediamine/H_2O_2 (OPD) substrate. The reaction was stopped as in the gp41 avidity study by adding 2 M H_2SO_4, a DSV measured and a DSV index calculated. High-avidity antibody controls were from asymptomatic cases, known to have been HIV^+ for longer than 12 months, whose CD4 count was high, whose clinical status and prognosis was good and who consistently gave low DSV readings ($x = 0{\cdot}2$). Prior tests showed that <5% of the p24 antigen was removed from the wells of plates by the DEA treatment.

Anti-p17 titres

Recombinant p17-GST (*E. coli*) (ADP633; MRC AIDS Repository) fusion protein was dissolved at 4 μg/ml in PBS pH 7·2 and 50 μl added to wells of ELISA grade high binding microtitre plates (Costar, High Wycombe, UK; code no. 3590) for 24–48 h at 8°C. Free solid-phase sites were blocked with PBS containing 2·5% w/v bovine serum albumin (BSA; Sigma-Aldrich Co. Ltd, Poole, UK) for 1 h at 37°C and the wells then washed four times with PBS–T. Patients' sera were inactivated by 1 : 10 dilution in PBS containing 0·55% Triton X-100 and 2·5% BSA. Subsequent dilutions were made in PBS–T containing 2·5% BSA, 0·1% Tween 20. The sera were tested in four-fold dilutions from 10^{-2} and reacted overnight with the solid phase at 8°C. After six washes, a routine ELISA was performed using rabbit HRP anti-human IgG (Dako, High Wycombe, UK; code no. P214) at 1 : 6000 and OPD substrates was added to wells for 15 min at 21°C. The colour reaction was stopped with 2·5 M H_2SO_4. The titre was the serum dilution which gave an Abs_{492} reading of 0·2.

Avidity index of anti-p17

The method of Chargelegue and colleagues [20–22] was used. Prior tests showed that 8 M urea had no effect on the solid-phase antigen. Each serum was tested at a predetermined dilution giving an $Abs_{492} = 1{\cdot}0$. Duplicate wells were then treated with 8 M urea or PBS for 12 min at 21°C. After six washes the bound IgG was measured as described above and the avidity index (AI) calculated as:

$$\text{AI} = \frac{\text{mean OD urea-treated wells}}{\text{mean OD untreated wells}} \times 100$$

AI values <30% were taken to mean mainly low-avidity-specific antibody.

p24 antigen

The p24 antigen assays were carried out, according to the kit instructions, using the Abbott HIVAG-1 test (no. 7042; Abbott Labs).

RESULTS

Antibody avidity

Sera from all cases of seroconversion/recent HIV illness were assessed for their relative anti-gp41 avidity. Figure 1 shows that where sequential sera were available, the avidity of anti-gp41 IgG increased following seroconversion, although the rate of increase was not the same in each case, with patients 2 and 3 increasing their avidity faster than patients 1 and 4. Table 1 shows that anti-gp41 avidity was low in early cross-sectional sera and high in later sera.

Thirty sera from cases of remote HIV infection were tested and all were found to have high-avidity anti-gp41 antibodies irrespective of the patient's clinical status.

Anti-p24 and anti-p17 avidity studies were carried out on sequential sera from patients 1, 2, 3, 5 and 7 and anti-p24 avidity studies on sera from patients 4, 6 and 8. In marked contrast to the anti-gp41 avidity results, high-avidity anti-p24 and anti-p17 developed during the follow-up period in only one of the four patients (patient 3, Fig. 1c) from whom we had several sequential sera. High-avidity anti-p24 was already present in the first of the two sera from patient 6, 11 months after a seroconversion illness (anti-p17 avidity not assessed). Results of anti-p24 and anti-p17 avidity tests in the remaining cross-sectional sera are shown in Table 1.

Although patient 1 did not mature his anti-p24 or anti-p17 avidity, there was a slight increase in avidity observed over the follow-up period (Fig. 1a), as there was also in the avidity of the anti-p24 antibodies in sera from patient 4 (Fig. 1d; anti-p17 avidity not assessed).

Ten of 45 sera from cases of remote HIV did not contain sufficient anti-p24 antibody for avidity testing. Of these, six were from cases of AIDS, two from cases of ARC, and two were from cases who were lost to follow up but asymptomatic at the time the specimens were taken. Twenty-one remote sera had a DSV index <5 (i.e. high-avidity antibody). None of these was from cases of ARC or AIDS. Fourteen sera had DSV indices >5 (low-avidity antibody); six were from asymptomatic patients, two from cases of ARC and six from cases of AIDS; all had high-avidity anti-gp41. Low-avidity anti-p24 antibodies were present in sera from 2 to 7

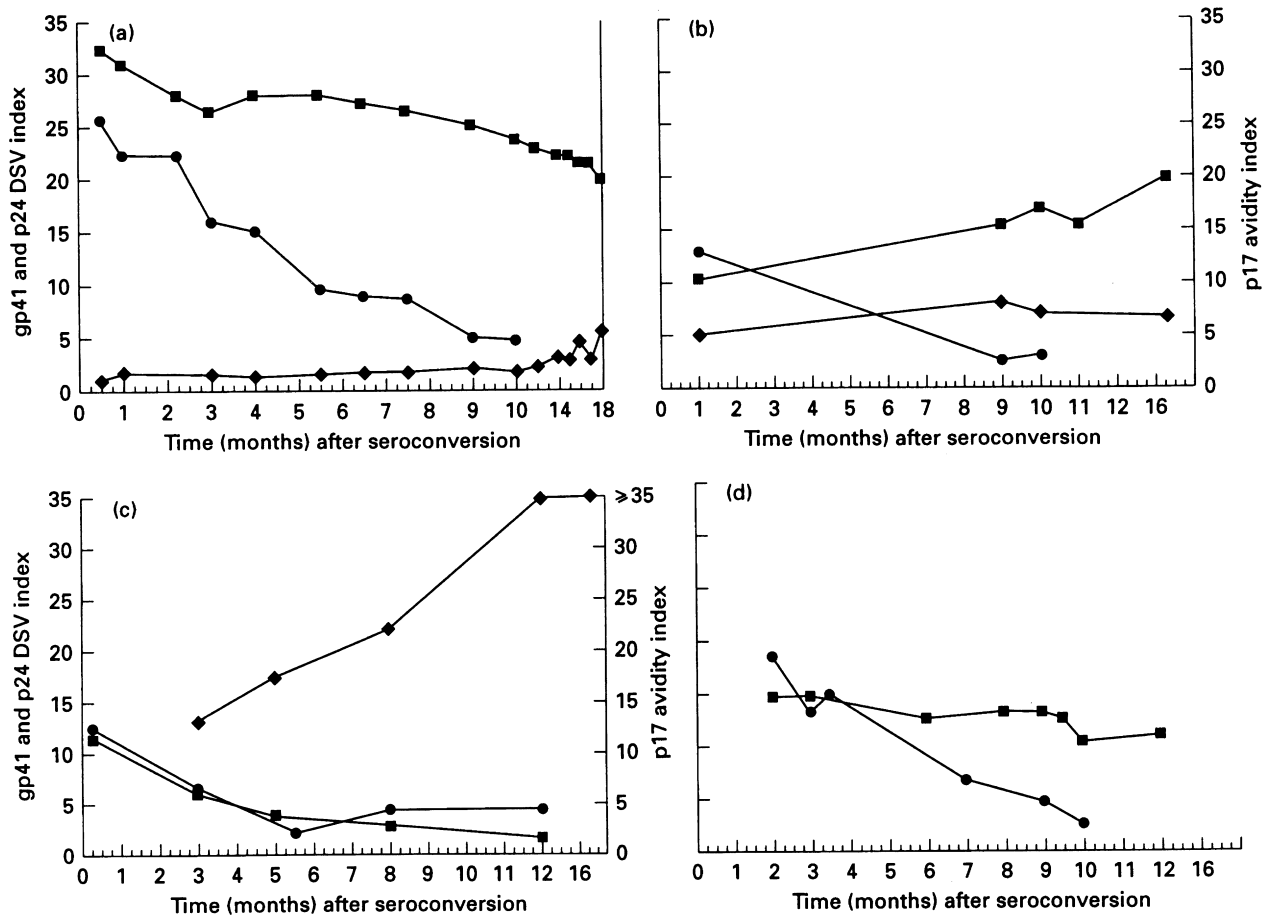

Fig. 1. The changes in the avidity of IgG antibodies specific for gp41, p24 and p17 in sequential sera from (a) seroconvertor patient 1, (b) seroconvertor patient 2, (c) seroconvertor patient 3, (d) seroconvertor patient 4. ●, gp41; ■, p24; ◆, p17.

months before the diagnosis of ARC or AIDS was made (first sera available) and persisted for up to 22 months after the diagnosis (last observation point).

Antibody titres

Patients 1–4 were followed in sufficient detail to ascertain when maximum antibody titres were reached. Table 2 summarizes when maximum antibody titres for each patient were reached and gives details of whether they were maintained or not.

High anti-gp41 titres were observed in all remote cases studied, including those with ARC or AIDS. However, detectable levels of anti-p24 antibody were seen in only 35 of the 45 remote sera

Table 2. The time taken for maximum antibody titres to be reached (and the maximum titres)

Patient	Anti-gp41	Anti-p24	Anti-p17
1	3 months ($>2 \times 10^4$) Maintained‡	2 months ($>5 \times 10^4$) Titre fell from 5·5months	3 months ($>1 \times 10^5$) Titre fell from 4 months
2	8 months* ($>2 \times 10^4$) Maintained‡	8 months* ($>5 \times 10^4$) Maintained‡	17 months† (6×10^3)
3	8 months ($>2 \times 10^4$) Maintained‡	5 months ($>5 \times 10^4$) Maintained‡	21 months† ($2{\cdot}5 \times 10^5$)
4	3 months ($>2 \times 10^4$) Maintained‡	3 months ($2{\cdot}8 \times 10^3$) Titre fell from 3·5 months	ND

* No sera available between 1 and 8 months post-seroconversion.
† Last serum taken.
‡ Antibody titre maintained over period of study.
ND, Test not done.

Table 3. CD4 and CD8 counts in sera from patients 1–4

Patient	Range ($\times 10^9/l$)	Mean ($\times 10^9/l$)	s.d.
CD4 counts			
1	0·08–0·53	0·322	0·091
2	0·28–0·82	0·52	0·149
3	0·48–0·80	0·63	0·099
4	1·144–2·251	1·534	0·37
CD8 counts			
1	0·06–2·26	1·181	0·575
2	0·77–2·05	1·435	0·547
3	0·68–1·97	1·282	0·429
4	0·673–2·073	1·178	0·532

examined. Anti-p24 titres in these sera ranged from 126 to $>5{\cdot}12 \times 10^4$. The anti-p24 titres fell in one case of remote HIV infection (from 794 to 126 in seven sera over a period of 9 months) but the avidity of the anti-p24 antibody remained high. So far this case has not progressed to ARC or AIDS (9 months after the last serum was taken and examined for avidity). In one other case, the anti-p24 titre has fallen from 1585 to 316, but this case was already classified as ARC (anti-p24 titre 1585 at the time) and then progressed to AIDS. The patient already had low-avidity anti-p24 when diagnosed as having ARC. Both these cases suggest that the low avidity of anti-p24 was more relevant to disease progression than antibody titre.

CD4 and CD8 counts

CD4 and CD8 counts ($\times 10^9/l$) from patients 1–4 are given in Table 3. Normal values for CD4 and CD8 counts were taken as $0{\cdot}83 \pm 0{\cdot}29 \times 10^9/l$ and $0{\cdot}56 \pm 0{\cdot}23 \times 10^9/l$, respectively [28].

p24 antigens was detected in sera from patients 1 and 2 only up to 7 days post-seroconversion. In patient 3, p24 antigen was detected only in sera taken up to the point at which the antibody tests were becoming positive and was no longer detectable when the first truly HIV antibody-positive serum was taken. The serum taken from patient 4, 2 months after his seroconversion illness, was equivocal for p24 antigen. A serum taken 1 month later was p24 antigen-negative. p24 antigen was detected in a serum from patient 10 14 days after his last HIV^- sample. p24 antigen was not detected in any of the other sera.

DISCUSSION

Relative avidity studies are useful for the differentiation between cases of primary and past infection or reinfection/reactivation in several viral infections [13,14,16,17,19,26,28], and it was hoped that they might provide a means of identifying cases of recent HIV infection. We therefore examined the relative avidity of anti-gp41, anti-p24 and anti-p17 antibodies in sequential and cross-sectional sera from cases of well-defined recent HIV seroconversion and of anti-gp41 and anti-p24 antibodies in sera from cases who have been HIV^+ for longer than a year. We believe this is the first study in which a comparison of the simultaneous maturation of the antibody response to more than one HIV antigen in patients immediately following HIV infection has been made.

Well documented and dated collections of sequential sera from cases of HIV infection immediately following seroconversion are difficult to obtain. Despite the small number of such cases to which we have had access, we have shown that only anti-gp41 avidity studies could reliably differentiate between cases of recent and 'remote' HIV infection. Anti-gp41 avidity increased in the months following seroconversion in all cases and remained high in cases of remote infection and ARC or AIDS. This confirms the findings of Radkowski *et al*. [23]. Antibody titres reached their maxima before anti-gp41 antibodies reached their highest avidity, thus limiting their ability to differentiate between recent and remote HIV infection. Our finding that once a maximum anti-gp41 titre has been achieved, like the anti-gp41 high avidity, it is maintained is in agreement with published work [31,32].

We have shown that the maturation of the humoral response to the gag proteins is very variable, and our studies reveal interesting differences between patients very early after seroconversion, confirming the findings of McRae *et al*. [24], who also showed that anti-p24 and anti-p17 avidity could not be used reliably to identify recent HIV infection. The variation in anti-p24 and anti-p17 antibody titres following recent infection with HIV also does not permit these perameters to confirm recent infection. However, whilst anti-gag avidity studies could not reliably differentiate between recent and earlier HIV infections, our anti-p24 avidity studies on sera from remote cases of HIV infection confirm the findings of Chargelegue and colleagues [20–22] that a decline in the avidity of anti-p24 or anti-p27 antibodies is a better predictor of the onset of AIDS than anti-p24 titre. Falls in anti-p24 titres before the onset of ARC/AIDS have been reported [30–32].

Our finding that high avidity anti-gag antibodies developed in some seroconvertors and were present in some remote asymptomatic cases shows that the avidity of anti-p17 and anti-p24 antibodies does increase with time, at least in some patients. We intend following patients 1, 2 and 4 to see if the avidity of their anti-p17 and anti-p24 antibodies does eventually become high. However, it would appear that the early rate of anti-p17/p24 avidity maturation might classify patients into 'fast' and 'slow' (or 'non') anti-p17/p24 avidity 'maturers'. High-avidity specific IgG antibody is known to be more protective and more efficient than low-avidity antibody in many biological reactions [15,33] and is indicative of a memory response. As all of our patients matured, or were in the process of maturing their anti-gp41 response, but only a few showed evidence of a maturing anti-p17 or anti-p24 response, it will be interesting to see if the delay in achieving, or inability to achieve, a high-avidity response against the p17 and p24 antigens early after seroconversion has any pathological significance.

The lower antibody avidity detected in many cases in the anti-p24 and anti-p17 assays compared with the anti-gp41 avidity assay is unlikely to be due to the slightly different methods used. Indeed, other avidity studies [13,14,17] have shown that the AI method used for the gag avidity work is less sensitive and detects low-avidity-specific antibody for a shorter period following seroconversion than the DSV method.

The production of high-avidity anti-gp41 by all patients in this study suggests that, irrespective of the number of circulating T cells, the components of the immune system required for a mature response were functionally effective with regard to gp41. However, there appeared to be a simultaneous impairment in the ability to mount a mature immune response against the p17 and p24 proteins in some, but not all, patients. This could not be explained in terms of total CD4 counts. The one patient who matured his anti-p17 and anti-p24 responses had lower CD4 counts (CD4 mean

$0{\cdot}603 \times 10^9/l$) than one of the three patients whose anti-p17 and anti-p24 responses did not mature (patient 4; CD4 mean $1{\cdot}534 \times 10^9/l$). Close examination of the CD8 counts and our avidity results and antibody titres also failed to detect any signs of correlation.

In conclusion, although the number of seroconvertors we have studied is small and consequently the conclusions we have been able to draw are limited, we have been able to establish that relative avidity studies do have an important role to play in HIV serology. As the detection of low-avidity anti-gp41 antibodies, indicative of recent infection, can be made with one serum and the establishment of rising titres requires at least two sera taken some days apart (and may be missed altogether), avidity studies can be of greater value in the serological confirmation of recent infection than antibody titres. We have clearly demonstrated that anti-gp41 avidity studies can be of greater value than anti-gp41 titres in the serological confirmation of recent HIV infection. However, in view of the inability of some patients to mature their anti-p17/p24 responses over the same time span, anti-p17/p24 avidity studies are not suitable for confirmation of recent HIV seroconversion. Unless a 'trend' which indicates falling anti-p17/p24 avidity can be detected in sequential sera [20,21,29], the presence of low-avidity anti-p17/p24 in a single serum is unlikely to be of value in the prediction of the onset of ARC/AIDS. A combination of anti-gp41 and anti-p17/p24 avidity results could be useful, however. A persisting low-avidity anti-p17/p24 response in the face of an increasing avidity anti-gp41 response (i.e. in a recent HIV seroconversion) may have some prognostic significance.

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