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Differential Regulation of the Antibody Responses to Gag and Env Proteins of Human Immunodeficiency Virus Type 1

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We have studied the antibody responses to Env and Gag antigens of human immunodeficiency virus type 1 (HIV-1) in several cohorts of HIV-1-infected individuals: long-term nonprogressors, progressors to disease, acute seroconvertors, and recipients of HIV-1 protease inhibitors. We conclude that the antibody responses to Env and Gag antigens are differentially regulated and that changes in the plasma viral load in the measurable range (500 to 10^8 RNA copies per ml) do not directly affect the antibody responses to these HIV-1 proteins. We provide quantitative estimates of HIV-1-specific immunoglobulin G concentrations in plasma, which can be in excess of 1 mg/ml for both anti-gp120 and anti-p24 once the immune response to HIV-1 has stabilized after seroconversion. We discuss the apparent paradox that the absence of anti-Gag antibodies (which have, at best, limited antiviral activity) is indicative of disease progression, while the retention of anti-Env antibodies (which do have antiviral activity) is of limited (or no) prognostic value. We show that the disappearance of anti-Gag antibodies during disease progression is highly unlikely to be due to immune complexing; instead, we believe that it reflects the loss of T-cell help that is more necessary for the anti-Gag than the anti-Env response.

Infection with human immunodeficiency virus type 1 (HIV-1) is associated with a vigorous humoral immune response to several viral antigens. Most HIV-1 proteins are immunogenic, but strong antibody responses are generated to the gp120 and gp41 envelope glycoproteins and the core/matrix proteins p24 and p17, encoded by the *env* and *gag* genes (3, 76). HIV-1, but not human T-cell leukemia virus type 1, infection is associated with hypergammaglobulinemia (13, 14), and although much of the excess antibody production is not virus specific, a large amount of it does recognize viral antigens.

The cellular immune response is probably dominant over the humoral in controlling HIV-1 replication during acute infection (49), but antibodies may reduce the rate of spread of HIV-1 between cells during the chronic phase (22, 39). Primary virus neutralizing antibody titers in plasma tend to be weak (62, 65, 66) and to develop only slowly (49, 59, 64). Only a limited or nonexistent correlation has been found between neutralizing antibody titers in vitro and disease progression (35, 38, 95, 99). However, two of three recent studies have found that long-term nonprogressors (LTNP) do have somewhat broader and more potent neutralizing antibody titers than disease progressors (19, 38, 62), indicating a potentially beneficial role of humoral immunity in slowing disease progression under some circumstances. It has also been reported that three HIV-1-infected individuals who did not develop an antibody response progressed unusually rapidly to AIDS and death (56, 71, 86, 93). These cases are anecdotal, in that there is no proof of a direct association between a failure to seroconvert and rapid progression, but they are also suggestive of a beneficial role for humoral immunity in delaying the onset of disease.

One clue to understanding the antibody response to HIV-1 is that it is not monomorphic: the antibody responses to Gag and Env antigens differ from one another. Although there is a wide range in the magnitude and specificity of the responses to different HIV-1 proteins, individuals who progress to clinical disease generally have lost (or have never acquired) the ability to develop an antibody response to Gag antigens. Indeed, multiple studies have shown that the early absence, or later loss, of an anti-Gag response is a poor prognostic indicator for the outcome of HIV-1 infection (17, 21, 41, 79, 81, 87, 100). In contrast, antibody responses to the gp120 and gp41 proteins tend to be strongly sustained throughout HIV-1 infection, sometimes diminishing partially or completely only as frank AIDS develops (21, 41, 87). That the magnitude of the antibody response to the envelope glycoproteins has no correlation with disease course, whereas a strong antibody response to Gag antigens indicates a good prognosis, presents a paradox: anti-Gag antibodies have few documented antiviral activities, while the envelope glycoproteins are the targets for essentially all well-characterized neutralizing antibody and antibody-dependent cellular cytotoxicity responses against HIV-1 (39, 67). Indeed, the potentially protective role of anti-Gag antibodies has been investigated by immunization of HIV-1-infected individuals with recombinant p17/p24 antigens (94) or with envelope-depleted virions (90), which resulted in little or no clinical benefit. In this report, we examine the anti-Gag and anti-Env antibody responses to HIV-1 infection, discuss the apparent paradox outlined above, and consider some of the implications that our observations may have for HIV-1 vaccine development. We conclude that the loss of the anti-Gag response during disease progression is not due to immune complex formation but more probably reflects the loss of T-cell help.

MATERIALS AND METHODS

Cohorts of HIV-1-infected individuals. Clinical information on the cohort of LTNP depicted in Fig. 1 has been published elsewhere (19). The patient designations in that report have been changed as follows: #2 to AD-18, #3 to AD-65, #4 to AD-63; #5 to AD-19; and #7 to AD-20. Three LTNP referred to as #6, #8, and #10 in reference 16 have since had a significant increase in plasma

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viremia and developed disease symptoms. They have now been reclassified as disease progressors AD-62, AD-64, and AD-66 and are included in that cohort in the present study. Other individuals in our progressor cohort have not been previously described and are designated AD-29, AD-61, AD-67, AD-68, AD-69, AD-70, AD-71, and AD-72. Progressing patients AD-1 and AD-2 have been described elsewhere, designated NYBC-A and NYBC-B, respectively (23). Longitudinal plasma samples from one additional LTNP (20086; AD-22) and one progressor (21083; AD-27) were obtained from Steven Wolinsky (Northwestern University, Chicago, Ill.). The acute seroconvertors AD-6, AD-11, AD-13, and AD-60 have been described elsewhere (49, 64). Individuals 406, 408, and 409 were participants in a phase I clinical trial of the protease inhibitor ritonavir (Abbot Laboratories, Inc., Chicago, Ill.). Individuals 408 and 409 were, when treatment was initiated, clinically and immunologically advanced with low CD4 cell counts, while individual 406 had been infected for approximately 3 years without showing disease symptoms. All plasma samples were inactivated by heating at 56°C for 1 h, and debris was removed by centrifugation (1 min, $14,000 \times g$).

Clinical assays. Plasma viral load measurements were made by using the branched-DNA (bDNA) assay of HIV-1 RNA (Chiron, Emeryville, Calif.) as described previously (18, 19). However, for AD-1 and AD-2 (Fig. 3), the viral load data are presented as the 50% tissue culture infectious dose per 10^6 $CD4^+$ cells and are derived from reference 23. Plasma immunoglobulin G (IgG), IgM, and IgA concentrations in some samples were determined by a commercial laboratory (SmithKline Beecham Clinical Laboratories, Syosset, N.Y.).

ELISA for antibodies to gp120, p24, p17, and gp41 peptide in plasma. Anti-gp120 titers were determined by antigen capture enzyme-linked immunosorbent assay (ELISA) as described previously (64, 66, 68). Briefly, gp120 from the MN strain (from the NIAID Reagent Repository) was diluted in Tris-buffered saline (TBS) containing 10% fetal calf serum and captured onto a solid phase (Immulon II plates; Dynatech, Chantilly, Va.) via adsorbed sheep antibody D7324 (Aalto BioReagents, Dublin, Ireland) to the carboxy terminus of gp120. The ELISAs for anti-p24 and anti-p17 used recombinant p24 and p17 expressed and purified as glutathione *S*-transferase fusion proteins. These proteins were coated directly onto microtiter plates by overnight incubation at 1 μg/ml in sodium bicarbonate buffer (pH 8.6). The anti-gp41 ELISA used an N-terminally biotinylated peptide (RILAVERYLKDQQLLGIWGCSGKLIC) corresponding to the most immunodominant continuous epitope of the gp41 ectodomain (Anaspec, San Jose, Calif.). This was captured (1 μg/ml in TBS) to the solid phase via avidin-DX (Vector Laboratories Inc., Burlingame, Calif.) which had been coated overnight in TBS (pH 7.4) at 10 μg/ml. As positive controls for the correct functioning of this assay, we showed that monoclonal antibodies 4D4 and 3D6 (gifts from Hermann Katinger, IAM Inc., Vienna, Austria) against this epitope reacted strongly with the biotinylated peptide.

In each assay, after washing away unbound antigen with TBS and blocking for 30 min with 2% nonfat milk powder in TBS, plasma samples were titrated by six serial threefold dilutions (initial dilution of 1:100 or 1:300, depending on the antigen) in a buffer containing 2% nonfat milk and 20% sheep serum in TBS. Bound antibody was detected by using alkaline phosphatase-conjugated anti-human IgG and the AMPAK system (Dako Diagnostics, Santa Barbara, Calif.). Midpoint titers were defined as the antibody dilutions giving half-maximal binding, after background subtraction, which was usually an optical density (OD) value of approximately 0.6 to 0.7. A reference plasma from individual AD-18 was used as a positive control on each plate, to ensure that titers were consistent throughout. When midpoint titers are below 1:1,000, their determination is subject to significant imprecision. To obtain more accurate profiles of antibody concentration changes over time in these cases, we selected a single plasma dilution (typically between 1:100 and 1:300) that contained a subsaturating concentration of antibody. The diluted serum was added to triplicate wells either containing or lacking antigen, and the mean OD values were calculated after subtracting the assay background (from wells with no antigen).

Competition ELISA for estimating plasma anti-p24 and anti-gp120 IgG concentrations. To measure the plasma concentrations of antibodies specific for gp120 and p24, the dilutions at which these antibodies approximate their average K_ds were first estimated from titrations of the plasma in the appropriate ELISA. This enabled us to use the plasma at known ratios of average antibody concentrations to K_d in a competition ELISA. In this assay, graded amounts of solution-phase antigen were used to compete off antibody binding to the solid phase. When plasma antibody is used at a concentration well in excess of its K_d, and the soluble antigen is present at a concentration similar to that of the plasma antibody, there is a substantial and calculable degree of complexing between the two molecules. In contrast, at lower antibody concentrations, the ratio of soluble antigen to K_d, not the amount of antibody, is the dominant factor in determining the degree of complexing.

Plasma, at a final dilution of 1:300 or 1:1,000, was incubated in solution with a range of concentrations of p24–glutathione *S*-transferase or MN gp120. The mixtures of antibody and antigen were immediately added to p24- or gp120-coated wells, so that antibody binding to both soluble and solid-phase antigen were allowed to approach equilibria simultaneously. Antibody binding to the immobilized antigen was then detected as described above. The mean OD values (background subtracted) were plotted as a function of the solution-phase antigen concentration. By solving the mass action equation that describes such a double equilibrium, we translated the degree of competition into the occupancy of soluble antigen on the paratopes (individual antigen-combining sites of the antibody; two per bivalent Ig molecule) (46). We were thus able to calculate approximate concentrations of the HIV-1 antigen-specific antibodies in the undiluted sera.

The principles that we have sketched so far were applied as follows. The OD values from the anti-gp120 and p24 titrations were plotted as a function of plasma dilution for samples from AD-62, AD-18, AD-60, and AD-6 (the individual plasma samples used from each of these individuals are indicated as open arrows in Fig. 2 and 4). Henceforth, what we refer to as the percent maximum OD has been corrected for the assay background OD (on solid-phase antigen) and then divided by the maximum (background corrected) OD value (at the plateau of saturation). Provided that the percent maximum OD is linearly related to occupancy on the antigen and that there is an excess of antibody over solid-phase antigen (so that the difference between the concentrations of free and total antibody can be neglected), the antibody concentration giving 50% of maximal OD should be threefold below that giving 75% of maximal OD (46). This theoretical value of 3 agreed to within a factor of 1.5-fold with the ODs of the plasma titrations that gave the values shown in Fig. 2 and 4. Therefore, at the plasma dilutions that gave half-maximal OD, it can be assumed that the concentrations of the gp120- or p24-specific antibodies could be approximated to their K_ds (46, 47).

We subsequently used the following derivations to calculate the plasma antibody concentrations: A = total paratope concentration; A_{free} = concentration of unoccupied epitope-binding sites on antibodies; B = total soluble antigen concentration; C = concentration of complex between paratope and epitope in solution; $K_{d(s)}$ = the dissociation constant for the binding of paratopes to epitopes on antigen in solution; E = solid-phase-anchored epitope-paratope complex per unit volume; F = total solid phase-anchored epitopes per unit volume; and $K_{d(sp)}$ = the dissociation constant for the binding of paratopes to epitopes on antigen that is anchored to solid phase. Then according to the law of mass action applied to double equilibria (47), $A_{\text{free}} = CK_{d(s)}/(B - C) = E K_{d(sp)}/(F - E)$.

We can choose a half-maximal relative occupancy of paratopes on the solid-phase antigen, that is, $E/F = 0.5$. Then $E/(F - E) = 1$, which gives $A_{\text{free}} = CK_{d(s)}/(B - C) = K_{d(sp)}$.

Furthermore, we let $A/K_{d(sp)} = D$, a dimensionless factor which is known from the anti-p24 and anti-gp120 titrations. The antigen in solution at half-maximal OD is in excess of that bound to the solid phase. Hence, the complex formation by solid-phase antigen is negligible in relation to that by antigen in solution: A_{free} is approximately equal to $A - C$. Henceforth, we calculate as if $A_{\text{free}} = A - C$.

We can now express $A - C = A/D$ and derive $C = A - A/D$, and then apply the law of mass action to the solution reaction alone: $(A - C)(B - C)/C = K_{d(s)}$.

The functional affinity for bivalent monoclonal antibody binding to solid phase is usually 1 order of magnitude higher than that for monovalent binding (48). A smaller difference for polyclonal sera could be explained if antibodies frequently bind bivalently to antigen molecules in solution that are already complexed by other noncompeting antibodies. In the current competition assays, there is potential for such polyvalent binding by antibodies of some specificities to antigen complexed by antibodies of other specificities. Hence, since the ratio $K_{d(s)}/K_{d(sp)}$ would be equal to 1 in the absence of antibody valency effects, and maximally around 10 with such effects active only on solid-phase binding, we have used a value of 5 for this ratio in order to take into account the potential for bivalent binding in solution. The 10-fold difference between the extremes of this factor would give a 5-fold difference in antibody concentration. This represents one factor of limited uncertainty in our conversions of competitor to antibody concentrations. A related one is the number of epitopes on the soluble antigen molecules that, on average, are simultaneously occupied at equilibrium.

After successive substitution of $K_{d(s)} = 5\ K_{d(sp)}$, $K_{d(sp)} = A/D$, and, for 50% competition, $C = A - A/D$ into $(A - C)(B - C)/C = K_{d(s)}$ and subsequent simplification, we get $A = B/6[1 - (1/D)]$.

If we choose 90% inhibition of antibody binding by the soluble antigen competitor, i.e., $E/(F - E) = 1/9$, and therefore $A_{\text{free}} = CK_{d(s)}/(B - C) = K_{d(sp)}/9$, which gives $C = A - A/9D$, then after substitutions into $(A - C)(B - C)/C = K_{d(s)}$ as above, we obtain $A = B/46[1 - (1/9D)]$.

RESULTS

LTNP have a low viral load and strong anti-Gag and anti-Env responses. Progression of HIV-1 infection toward disease has been reported to be associated with the loss of the anti-Gag, but not anti-Env, antibody response (17, 21, 41, 79, 81, 87). We confirmed this by using samples from 11 progressors and 5 LTNP (Fig. 1a), selecting the latest sample available from each individual at the time the comparison was performed (which is not necessarily the latest sample recorded in longitudinal profiles described below). The LTNP have much lower plasma viral loads than the progressors, as determined by either viral RNA (Fig. 1a) or infectious titer (not shown) assays; indeed, plasma viremia in several LTNP was undetect-

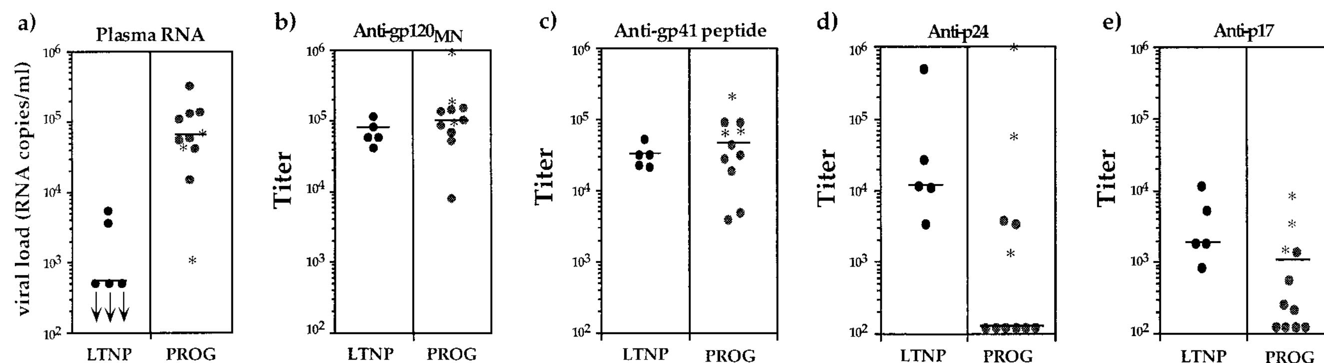


FIG. 1. Anti-Gag and anti-Env antibody titers and viral load in LTNP and progressors. Each individual in the cohorts is represented (●). All data were derived from the same time point. Individuals AD-62, AD-64, and AD-66, who progressed during our period of sampling, and were therefore reclassified from LTNP to progressor (PROG) status, are indicated (*). Plasma viral RNA was measured by using the bDNA assay. Median viral RNA copies per ml and median midpoint antibody titers are indicated by bars. Samples from all LTNP and progressors described in Materials and Methods were used, except for AD-1 and AD-2.

able by the bDNA assay and so is below 500 RNA copies per ml.

We found no significant difference in the median anti-gp120 midpoint titers of the progressor and LTNP cohorts (Fig. 1b), which were generally in the range 1:50,000 to 1:150,000 in both cohorts. This finding is consistent with our general experiences on a wider range of HIV-1-infected individuals; we rarely observe titers of <1:10,000, and we define these as low. Like the anti-gp120 response, anti-gp41 peptide titers were high in both the LTNP (mean around 1:50,000) and progressor (mean around 1:30,000) groups, although the variation in the anti-gp41 response was much greater in the progressor group (Fig. 1c). In marked contrast to the similarity in the anti-Env titers between the two cohorts, the anti-Gag antibody titers in the progressor cohort were clearly lower, on average, than in the LTNP (Fig. 1d and e). The range of anti-p24 titers observed was considerable, from <1:100, which is essentially undetectable, to almost 1:1,000,000. We consider anti-p24 titers of >1:5,000 to be high, and such titers are usually found only in LTNP (Fig. 1d). Of note was that no LTNP lacked a measurable anti-p24 response, while 6 of 11 progressors did. Anti-p17 titers tended to be about 1 log lower than anti-p24 titers, but the pattern of responses observed was similar to that for anti-p24: high titers in LTNP and low ones in progressors (Fig. 1e). One progressing individual (AD-62) had an exceptionally high anti-p24 titer of nearly 1:1,000,000; the unusual features of AD-62 are discussed below, but he and two other individuals progressed to disease symptoms while under study. The high anti-Gag titers shown derive from samples taken during the transitional period, and so they are unrepresentative of the situation in individuals who have progressed to disease.

We also determined total plasma IgG, IgM, and IgA concentrations in five LTNP and five progressors (Table 1, assays A and B). The plasma samples used for one of the LTNP and one of the progressors are indicated by closed arrows in Fig. 2. None of the LTNP was hypergammaglobulinemic (Table 1, assay A), indicating that the vigorous B-cell response to HIV-1 antigens in these individuals was virus specific. In contrast, two of the six progressors tested were hypergammaglobulinemic (Table 1, assay B). This finding is consistent with reports that HIV-1 infection is often, but not invariably, associated with elevations in plasma IgG of up to twice the normal level of about 16 mg/ml (13).

Longitudinal studies in progressors and LTNP. Static observations do not resolve whether progressing individuals develop an anti-Gag response and then lose it or whether they never develop such a response. To determine the temporal relationship between antibody responses and plasma viral load, we performed longitudinal studies (Fig. 2 and 3). Profiles not depicted were similar but usually less detailed in terms of numbers of samples available.

In both LTNP and progressors, anti-gp120 and anti-gp41 responses segregated together temporally, as did anti-p24 and anti-p17 responses, but the anti-Env and anti-Gag responses were distinguishable from each other in progressors (Fig. 2 and data not shown). In LTNP, anti-Env and anti-Gag titers were steady and high during the study, and viral loads were stable and low (Fig. 2a to c). Viral loads in progressors were usually high but fluctuated sometimes, while anti-Env titers were generally stable and high (Fig. 2d and f). AD-29 was atypical in that his anti-gp120 titer was the lowest in the group and his anti-gp41 response was also weak (Fig. 2d). Most progressors

TABLE 1. Concentrations of IgA, IgM, and IgG in plasma samples from LTNP and progressors[a]

Assay	Group	Concn (mg/ml)			Viral load (RNA copies/ml)
A	LTNP	IgG	IgM	IgA	
	AD-18	13.3	0.8	1.4	
	AD-19	15.5	1.3	0.6	
	AD-63	11.5	1.2	1.6	
	AD-65	15.8	0.9	3.2	
	Mean	14.0	1.1	1.7	
B	Progressors	IgG	IgM	IgA	
	AD-28	17.3	ND	ND	
	AD-62	36.4	5.5	0.9	
	AD-64	16.7	2.1	1.2	
	AD-66	18.1	1.1	1.6	
	AD-67	23.0	ND	ND	
	AD-68	17.3	ND	ND	
	Mean	21.5	2.9	1.2	
C	AD-62	IgG	IgM	IgA	
	Day 244	32.1	3.3	1.1	6,481
	Day 606	33.6	3.8	1.1	13,370
	Day 827	32.3	5.5	0.8	242,700
	Day 1050	22.6	1.2	0.7	<500

[a] The dates of analysis of samples from AD-18 and AD-62 are marked with closed arrows in Fig. 2. Assay C records longitudinal data from AD-62, who was reclassified from LTNP to progressor during this period. It should be noted that he initiated combination drug therapy at day 827. Plasma viremia is also recorded for AD-62. Note that hypergammaglobulinemia preceded the increase in viral load. ND, not done.

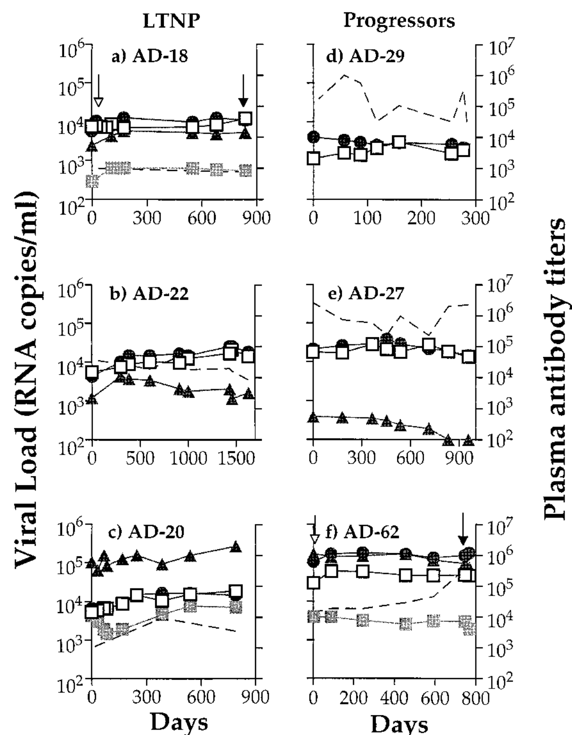


FIG. 2. Longitudinal antibody titers and viral load in LTNP and progressors. Results for anti-gp120 (●), anti-gp41 (□), anti-p24 (▲), anti-p17 (■), and plasma RNA (---) are shown. When anti-p24 or anti-p17 titers were consistently at the lower limits of detection (1:100), the data were omitted for clarity. Anti-p24 data for AD-27 are presented as mean ($n = 3$), net OD values (×1,000) at a plasma dilution of 1:200. Closed arrows indicate when plasma Ig concentrations were determined (Table 1), and open arrows indicate samples when anti-gp120 and/or anti-p24 antibody concentrations were estimated (Fig. 6; Table 2). Day 0 denotes when each individual entered the study.

had low (or undetectable) anti-Gag titers, exemplified by AD-29 (Fig. 2d). Either his anti-Gag response was lost before entering our study or else he never developed this response. However, AD-27 did develop an initial, weak anti-p24 response but lost it during the study (Fig. 2e), as did some other progressors (see below).

AD-62 is unusual, for he was initially considered to be an LTNP. He was infected with HIV-1 at least 7 years prior to the availability of the first clinical sample (day 0 in Fig. 2f). However, about 2 years after we began monitoring AD-62, his viral load increased from 5×10^3 to 10^5 copies/ml, he developed clinical symptoms of immunodeficiency, and he was reclassified as a progressor. His antibody titers to all four Gag and Env antigens were extremely high (especially the anti-p24 titer [see above]), but his anti-Gag titers had started to decline in the last available sample, taken after his viral load had risen (Fig. 2f). Of note was that hypergammaglobulinemia was one of the earliest indications of a deterioration in AD-62's clinical condition, as it was observed as early as day 244, well before the steep increase in viremia began around day 600 (Fig. 2f, Table 1, assay C). Unfortunately, the IgG content of earlier samples could not be determined, as insufficient material was available. AD-62 was subsequently treated with triple-drug therapy (nelfinavir, lamivudine, and zidovudine), initiated on day 827. By day 1050, viral RNA had dropped below assay detection limits (<500 copies/ml), and there was a significant reduction in plasma IgG from 32.3 to 22.6 mg/ml, close to the normal range. Whatever caused the hypergammaglobulinemia seems to be reversible by antiviral therapy. However, further monitoring of the natural progression of AD-62's antibody responses will not be possible because of antiviral drug use.

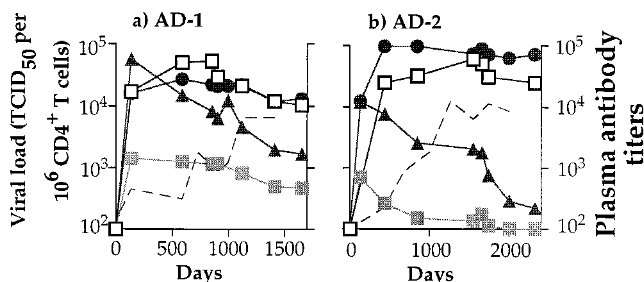


FIG. 3. Comparison of anti-Gag and anti-Env titers with viral load in two progressors. Day 0 is the time of the last sample taken prior to documented HIV-1 infection. Death occurred soon after the final time point in each case. Results for anti-gp120 (●), anti-gp41 (□), anti-p24 (▲), anti-p17 (■), and plasma RNA (---) are shown. Anti-p17 measurements are presented as mean ($n = 3$), net OD values (×1,000) at a serum dilution of 1:200.

We also studied two additional individuals from whom samples were available from seroconversion to AIDS and death. AD-1 seroconverted to gp120, gp41, p24, and p17 and died about 5 years after HIV-1 infection (Fig. 3a). His anti-gp120 titer was stable until death, while his anti-gp41 response was high (1:50,000) for 2 years and then gradually declined to a titer of 1:10,000 as disease developed. In contrast to his stable anti-gp120 response, AD-1's anti-p24 titer was very high (1: 50,000) 6 months after infection but declined slowly and inexorably over the next 4 years, until death. Only AD-1 ever developed a weak anti-p17 response, but it too gradually declined with time.

After seroconversion, anti-gp120 and anti-gp41 titers in AD-2 remained high (around 1:90,000 and 1:50,000, respectively) and stable for 7 years until death. However, an initially robust anti-p24 response (1:10,000) was followed by a slow decline to a titer which remained constant at an intermediate level (1:2,000) for about 2.5 years. A precipitate decline in anti-p24 to almost undetectable levels then preceded death. Anti-p17 closely paralleled the anti-p24 response (Fig. 3b).

The development of antibody responses in acute seroconvertors. The studies described above monitor the antibody responses to HIV-1 over a period of years. To gain insight into the rates at which antibody responses to different HIV-1 antigens initially develop, we studied four individuals undergoing primary infection and seroconversion to HIV-1 (Fig. 4). Each presented within weeks of sexual exposure to HIV-1, some with symptoms indicative of primary infection syndrome (43). Note that day 0 corresponds to the date of presentation, not of infection. In general, the kinetics of the anti-gp41 and anti-gp120 responses were similar, although anti-gp41 was usually detected earlier than anti-gp120, probably because the anti-gp41 assay is more sensitive. Likewise, anti-p17 and anti-p24 responses generally segregated together, the lower anti-p17 titers probably reflecting the lesser immunogenicity of p17.

(i) Seroconvertor AD-11. AD-11 presented with a very high viral load (10^8 RNA copies/ml) prior to seroconversion (Fig. 4a). His viral load subsequently declined over the next 200 days, to a relatively stable level of about 10^4 copies/ml. AD-11 seroconverted to gp120 on about day 23 after presentation, and an anti-gp120 titer was first quantifiable on day 58. This titer then increased steadily until about day 430, after which it remained constant in the 1:40,000 to 1:50,000 range until day 894. Seroconversion to the gp41 peptide was detectable on day 10, and the anti-gp41 titers plateaued in the 1:20,000 to 40,000

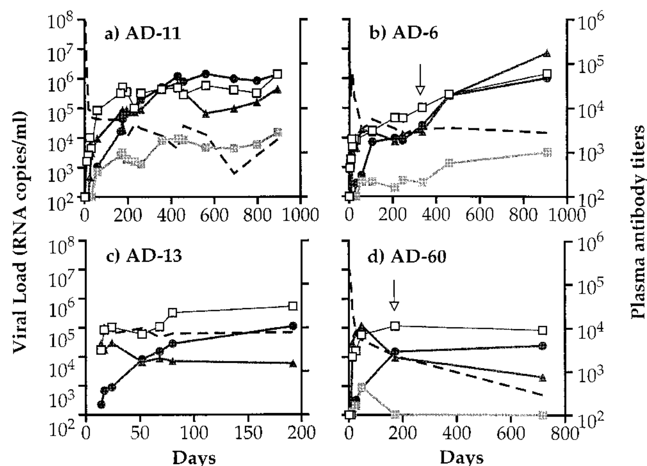


FIG. 4. Comparison of anti-Gag and anti-Env responses with viral load in four seroconvertors. Results for anti-gp120 (●), anti-gp41 (□), anti-p24 (▲), anti-p17 (■), and plasma RNA (---) are shown. For individuals AD-6, AD-11, and AD-60, anti-p17 measurements are presented as mean (n = 3), net OD values (×1,000) at a serum dilution of 1:200. Anti-p17 antibodies were not detectable in AD-13. Open arrows indicate samples for which anti-gp120 and/or anti-p24 antibody concentrations were estimated (Fig. 6; Table 2). Day 0 denotes when each individual entered the study.

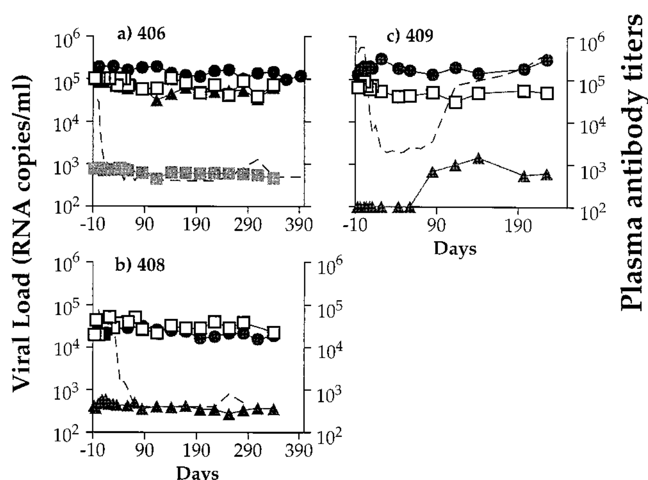


FIG. 5. Comparison of anti-Gag and anti-Env responses with viral load in three protease inhibitor recipients. Ritonavir was first administered on day 0. Results for anti-gp120 (●), anti-gp41 (□), anti-p24 (▲), anti-p17 (■), and plasma RNA (---) are shown. Anti-p24 and anti-p17 measurements in patients 408 and 409 are presented as mean (n = 3), net OD values (×1,000) obtained at a serum dilution of 1:300.

range on day 167 (rather earlier than the anti-gp120 response stabilized) and then also remained steady until day 894. An anti-p24 response was detectable on day 23. The anti-p24 titer rose gradually until day 167 and then remained stable (around 1:9,000) for at least 96 days. A further fourfold increase in the anti-p24 titer then occurred, to a maximum on day 430, before a decrease to a minimum titer of 1:7,000 on day 561, followed by another increase. The temporal pattern of the anti-p17 response closely mirrored that of the anti-p24 response, indicating that the oscillations in these titers were not artifactual.

(ii) Seroconvertor AD-6. AD-6 was also highly viremic on presentation (5×10^6 RNA copies/ml), and as for AD-11, his virus load declined over 6 months to a steady state of around 10^4 copies/ml (Fig. 4b). AD-6 seroconverted to gp41, gp120, p24, and p17 on days 2, 4, 8, and 23, respectively. His anti-gp41 response rose sharply over the first 32 days and then gradually increased to a titer of 1:60,000 by day 909. Anti-gp120 titers rose steadily until day 106 and remained constant until day 336, while the anti-p24 and anti-p17 responses increased until day 57 and then also remained constant until day 336. After day 336, there were large increases in titers to all four proteins, the anti-gp120 increase being >10-fold, the gp41 peptide being about 6-fold, and the anti-Gag increases being about 50-fold. The maximum anti-p24 titer recorded on day 909 was 1:175,000, which is exceptionally high (as for the anti-p17 response), whereas the anti-gp120 and anti-gp41 titers of 1:48,000 and 1:60,000, respectively, were in the normal range. No changes in plasma viremia were associated with the changes in antibody titers between days 336 and 909.

(iii) Seroconvertor AD-13. AD-13's viral load was around 10^5 copies/ml when the first sample became available 14 days after initial presentation on day 0, and only a modest decline in viremia then occurred (Fig. 4c). AD-13 had already seroconverted to gp120, gp41, and p24 by day 14 but never seroconverted to p17. His anti-gp120 titer increased steadily between days 14 and 192. The anti-gp41 titer was already 1:6,000 on day 14 and then remained at about 1:10,000 from days 17 to 68 before increasing further to 1:31,000 by day 192. In contrast to the gradually rising anti-Env responses, AD-13's anti-p24 titer was at its highest on day 14 (1:4,000) and then declined slowly to a low titer of 1:1,500 by day 192.

(iv) Seroconvertor AD-60. Like AD-6 and AD-11, AD-60 presented with a high viral load (10^7/ml) which declined over the next 6 months (Fig. 4d). AD-60 was antibody negative on day 7 but had seroconverted to gp120, gp41, p24, and p17 by day 13 (Fig. 4d). His anti-gp120 response rose gradually to a low titer of 1:2,900 on day 171 and remained around that level until day 717. The anti-gp41 response behaved similarly, although it stabilized more rapidly at a titer of about 1:10,000 by day 47. In contrast to the anti-Env responses, the anti-p24 and anti-p17 titers rose between days 13 and 47 (maximum p24 titer of 1:11,400) but then declined significantly. The anti-p17 response was no longer detectable after day 171, and the anti-p24 titer continued to decline until day 171. This deterioration in the anti-Gag response was not associated with an increase in plasma viremia.

Antibody responses in recipients of protease inhibitors. Studying acute seroconvertors allowed us to compare the development of the antibody responses to different antigens and the relationship of this process to changes in viral load. However, treatment of HIV-1-infected individuals with potent antiretroviral agents permits examination of the effect on antibody titers of a relatively rapid perturbation of a stable virus load. The protease inhibitor ritonavir was therefore administered to three patients, in each of whom plasma viremia was rapidly and effectively suppressed to below the threshold of the assay (500 RNA copies/ml). In two recipients, the reduction of viremia was sustained for a year; in the other, viremia soon rebounded. This was due to the development of drug-resistant mutants (data not shown).

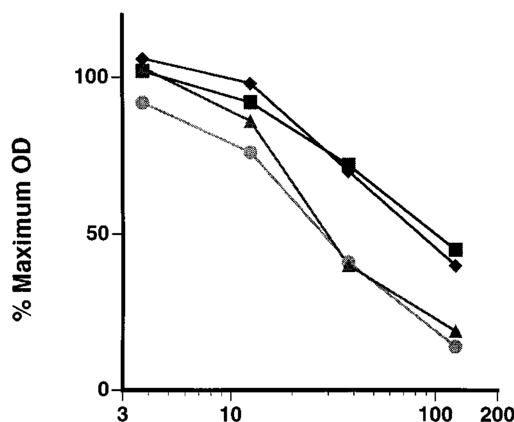
(i) Patient 406. Patient 406 had initially very strong antibody responses to Env and Gag antigens (Fig. 5a). His anti-gp120 titer of approximately 1:200,000 was above average, and his anti-gp41 titers of 1:50,000 to 1:100,000 were normal. The anti-p24 titers were high (around 1:50,000), and anti-p17 was detectable, albeit at a low titer (1:500). The rapid and sustained decline in plasma viremia caused by the protease inhibitor was not associated with a significant change in antibody titers to any Env or Gag antigen over a 1-year period.

(ii) Patient 408. The antibody responses of patient 408 were initially rather weak (Fig. 5b). His anti-gp120 titer (1:25,000) was below average, although anti-gp41 was in the normal titer range. The anti-p24 response was too low to titer accurately (anti-p24 ELISA OD values in the range indicated approximate to titers of around 1:300). Consistent with this, no anti-p17 response was detectable. Despite the rapid and sustained decline in plasma viremia caused by the protease inhibitor, no significant changes in the antibody titers to Gag and Env antigens occurred within one year.

(iii) Patient 409. Patient 409 had strong anti-gp120 and anti-gp41 titers in the 1:200,000 range, but anti-p24 and anti-p17 were undetectable (Fig. 5c). This was consistent with patient 409's advanced disease state (not shown). While the anti-gp120 and anti-gp41 titers remained constant throughout the duration of protease inhibitor therapy, a partial restoration of the anti-p24 response was detectable from day 43 onward. An anti-p24 titer of around 1:1,000 was achieved by day 85, and the p24 antibodies remained detectable until at least day 290. There was, however, only a minimally detectable increase in the anti-p17 response (not shown).

HIV-1-specific plasma antibody concentrations. We estimated the absolute concentrations of anti-p24 and anti-gp120 antibodies in selected plasma samples by a competition ELISA, using known amounts of the cognate antigen. The four samples used, from one LTNP, one progressor, and two seroconvertors, are indicated by open arrows in Fig. 2a and f and Fig. 4b and d. The competition curves show the decreases in binding of plasma antibody to solid-phase antigen that are caused by increasing amounts of soluble competitor antigen (Fig. 6). From these binding curves, the concentrations of competitor antigen causing 50% and (where possible) 90% inhibition were determined and used to calculate plasma anti-p24 and anti-gp120 concentrations as outlined in Materials and Methods.

The derived concentrations of anti-p24 and anti-gp120 ranged from 370 to 1,600 μg/ml for anti-gp120 (Table 2, assay A) and from 82 to 1900 μg/ml for anti-p24 (Table 2, assay B). Whenever it was possible to obtain both 50 and 90% inhibition of antibody binding by added antigen, very similar concentration estimates were obtained in each case. In addition, one sample (AD-18) was tested in separate competition assays at two dilutions (1:300 and 1:1000), and similar derived plasma antibody concentrations were obtained (Table 2, assay A). These internal controls serve to provide some validation of the method. The estimated average K_d values of the plasmas were rather low. This could be indicative of an underestimation of the IgG concentrations, because there are an unknown number of epitopes simultaneously occupied on antigen in solution which could not be accounted for when this parameter was calculated. Thus, if anything, our estimates of HIV-1-specific IgG concentrations are likely to be minimum estimates rather than overestimates.



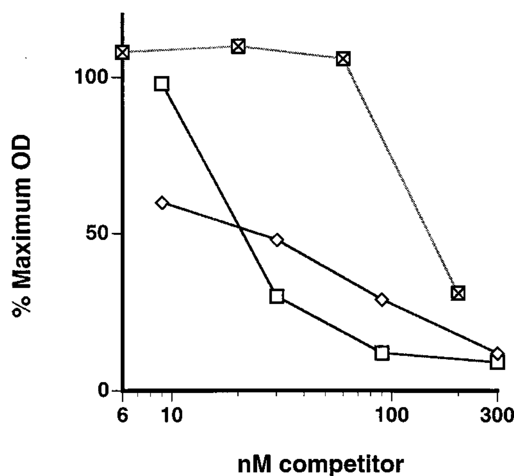


FIG. 6. Estimation of the concentrations of anti-gp120 and anti-p24 antibodies by antigen competition ELISA. Plasma samples (and their dilutions): (a) AD-18 (an LTNP) (1:300) (■); AD-18 (1:1,000) (●); AD-6 (a seroconvertor) (1:1,000) (▲); AD-60 (a seroconvertor) (1:1,000) (◆); (b) AD-18 (1:300) (□); AD-62 (a hypergammaglobulinemic progressor) (1:1,000) (⊠); AD-60 (1:300) (◇). The individual plasma samples used are indicated by open arrows in Fig. 2 and 4. They were as follows. AD-18 is a stable nonprogressor; AD-62 was sampled at a time when viral load was rising during progression. The seroconvertor AD-6 was sampled when the antibody response had not reached full maturity, and seroconvertor AD-60 was sampled when anti-gp120 had reached a stable but low titer, whereas the anti-p24 response was already beginning to drop.

DISCUSSION

Our studies of LTNP and progressors confirm that loss of an anti-Gag response is a poor prognostic indicator, whereas anti-Env responses are retained as disease progresses. To gain insight into why this is so, we studied several groups of patients.

From acute seroconvertors, we concluded the following: (i) Anti-Env and anti-Gag responses are independently regulated in terms of both kinetics and magnitude. Evidence includes the following: for AD-11, oscillations in anti-Gag titers occur when anti-Env titers are relatively constant; for AD-6, the anti-Gag titer was constant for 49 days between days 57 and 106, while the anti-gp120 titer increased sixfold; for AD-13, there was no temporal relationship between the development of the anti-gp120 and anti-p24 responses; and for AD-60, titers to gp120 increased simultaneously with decreases in titers to p24 and p17.

(ii) The anti-Gag decline can occur very soon after infection (in AD-60, and to a lesser extent in AD-13, within 6 months) and is not associated with parallel changes in anti-Env. Neither AD-13 nor AD-60 ever developed a strong (>1:10,000) anti-p24 titer, and no anti-p17 was ever detected in AD-13. Whatever (irreversible?) immunological events lead to an inability to generate a strong, sustained anti-Gag response, they can occur rapidly. This finding emphasizes the importance of starting antiviral therapy early.

(iii) Anti-Env and anti-Gag titer increases and decreases are unrelated to changes in plasma viremia in the measurable range. In AD-11, the anti-Gag oscillation between days 261

TABLE 2. Estimations of anti-gp120 and anti-p24 antibody concentrations in LTNP and seroconvertor samples[a]

Assay	Plasma	Antigen	Final plasma dilution	Plasma dilution at K_d	D	% Competition	Soluble competitor concentration (nM)	Paratope concn in diluted plasma (nM)	IgG concn in undiluted plasma (μM)	IgG (μg/ml)	Avg K_d (nM)
A	AD-18	gp120	1/1,000	1/23,000	23	50	28	4.9	2.4	370	0.11
	AD-18	gp120	1/300	1/23,000	77	50	100	17	2.5	380	0.11
	AD-60	gp120	1/1,000	1/2,900	2.9	50	83	21	11	1,600	3.7
	AD-6	gp120	1/1,000	1/4,100	4.1	50	30	6.6	3.3	500	0.81
B	AD-62	p24	1/1,000	1/1,000,000	1,000	50	150	25	12	1,900	0.012
	AD-18	p24	1/300	1/8,300	28	50	21	3.6	0.54	82	0.066
	AD-18	p24	1/300	1/8,300	28	90	200	4.5	0.68	100	0.082
	AD-60	p24	1/300	1/2,100	7.0	50	24	4.7	0.70	100	0.33
	AD-60	p24	1/300	1/2,100	7.0	90	330	8.4	1.3	190	0.60

[a] An explanation of how the antigen-specific IgG concentrations were calculated is given in Materials and Methods.

and 561 was independent of alterations in viral load; in AD-6, viremia was steady when exceptionally large increases in anti-Gag and anti-Env titers occurred after day 336; in AD-13 and AD-60, the anti-Gag declines were not associated with viral load changes. Anti-simian immunodeficiency virus titers in macaques infected with virulent and attenuated simian immunodeficiency virus strains were also similar, despite large differences in viremia (70).

The lack of relationship between antibody titer and virus load was also apparent in three chronically infected protease inhibitor recipients; sustained reductions in plasma viremia caused no changes in anti-gp120. In patient 409, partial restoration of the anti-p24 response during the period of low viremia took only a few weeks, which is encouraging for the prospects of restoring some immune function after aggressive therapy (44). The viremia decrease in patient 409 was only transient (due to drug resistance), but when it rebounded, the restored anti-Gag response was unaffected for several months. This is not consistent with the idea that Gag antibodies appear in plasma because they are released from immune complexes, and vice versa. Furthermore, the anti-p24 titer increase in patient 409 preceded the rise in viremia and so is unlikely to be driven by the increase in plasma antigen.

Several mechanisms have been proposed to explain why anti-Gag responses decay when anti-Env responses are sustained. The most common is that anti-p24 is removed by immune complexing with excess p24 protein (6, 27, 28, 50, 69). It is not obvious why anti-gp120 would not be subject to the same fate, but one explanation put forward is that p24 is more abundant than gp120 in plasma and that anti-p24 is less abundant than anti-gp120. There are serious concerns about the validity of this idea. Total p24 concentrations in plasma very rarely exceed 1 ng/ml, and some of this protein is particulate and thus so unavailable for antibody complexing (17). A high viral load of 10^6/ml corresponds to a particulate p24 content of only 50 pg/ml, so even if there were a 100-fold excess of soluble over particulate p24 (17), there would still be only 5 ng of circulating p24 antigen per ml. Plasma gp120 and p24 estimates are severely affected by the presence of cognate antibodies. However, Gag and Env proteins are synthesized in comparable amounts in HIV-1-infected cells. Although virions contain more p24 than gp120 molecules (ca. 1,200 and 200, respectively, corresponding to 5×10^{-17} g of each protein), the p24 is within the core and released only after virolysis or cytolysis (17), whereas gp120 is readily shed from virions and infected cells (32, 60). Overall, it is hard to believe that free p24 available in plasma for antibody complexing greatly exceeds free gp120; both are probably present in the picogram/milliliter to low nanogram/milliliter range.

What are the plasma concentrations of HIV-1-specific antibodies? We estimated that plasma anti-gp120 concentrations were in the range 0.1 to 1.0 mg/ml once the immune response matured (64). To confirm and extend this observation, we developed another assay (based on antigen competition) for determining anti-gp120 and anti-p24 levels. Our new estimates for plasma anti-gp120 are consistent with our previous ones; the anti-gp120 content in the day 336 sample from seroconvertor AD-6 was previously estimated to be around 400 μg/ml (64), and we now provide a value of 500 μg/ml for this sample (Table 2). Overall, our estimates on seroconvertor and LTNP plasma samples range from 370 to 1,600 μg/ml for anti-gp120 and from 82 to 1,900 μg/ml for anti-p24. Since the plasma IgG concentration is about 20 mg/ml (Table 1) (13), HIV-1-specific antibodies comprise a significant fraction of the total plasma IgG. This conclusion is consistent with estimates of HIV-1-specific B-cell frequencies. For example, 2 to 17% of IgG-secreting cells from HIV-1-infected individuals were found to be gp160 specific and 1 to 9% were found to be p24 specific (82), which would place plasma anti-HIV-1 concentrations in the milligram/milliliter range (3 to 26% of 20 mg/ml). Individual B-cell clones contribute on average about 40 μg of gp120-specific antibody per ml to plasma (37), and there are many B-cell epitopes on gp120 (11, 68), also implying that anti-gp120 is likely to approach the mg/ml range.

An antigen competition assay is subject to inaccuracies when used to analyze polyclonal sera and bivalent antibodies, but we believe that it is accurate to within an order of magnitude. This degree of precision is acceptable for the present study, since the amounts of anti-HIV-1 in plasma (milligram/milliliter range) exceed HIV-1 antigen concentrations (nanogram/milliliter range) by up to 10^5-fold. A perspective on these values is that the production of 10^{10} virions per day corresponds to ca. 1 μg of viral protein (40, 97), which contrasts starkly with the presence of at least 5 g of HIV-1-specific antibodies in plasma (a conservative estimate of 1 mg of these antibodies per ml, in a plasma volume of 5 liters).

It has also been suggested that anti-Gag and anti-Env responses are differentially sensitive to circulating Gag and Env antigen concentrations and that there is insufficient Gag antigen to stimulate antibody production under some circumstances (41). In principle, shedding of gp120 from viruses or virus-infected cells, combined with its external location, could increase the antigenicity of this protein by raising its effective concentration, while the internal location of Gag proteins

could make them relatively inaccessible to the immune system. However, much circulating p24 is present in nonparticulate form (17), and the destruction of up to 10^9 infected $CD4^+$ T cells each day in progressing patients (40) would surely liberate sufficient p24 to sustain an antibody response. Furthermore, the absolute amounts of p24 and gp120 in plasma are probably not very different (see above).

Our preferred explanation, as others have also argued (6, 41, 81, 89), is that the presence of anti-Gag antibodies is simply a surrogate marker for an efficiently functioning immune system. T-cell help is required for the efficient presentation of antigens to B cells, and perhaps also to maintain antibody secretion from terminally differentiated plasma cells (51–54, 84). Thus, the anti-Gag loss as disease progresses could be due to the inexorable destruction of $CD4^+$ T-helper cells through HIV-1 infection. But this raises another paradox: a global loss of T-cell help could not account for the retention of the anti-gp120 response, unless the anti-Env and anti-Gag antibody responses have differential requirements for T-cell help. We believe that this may, in fact, be the case. The obvious problem is, of course, that gp120 does not have the characteristics of a typical T-independent antigen, which tend to be polysaccharide or repetitive in nature (45). Neither does the sustained, high-level IgG response to gp120 observed during HIV-1 infection resemble a T-independent antibody response; the latter has been considered to be generally transient, weak, and IgM mediated (45).

Several factors affect the possibility of a relatively T-independent anti-gp120 response. (i) gp120 binds CD4; Gag proteins do not. The CD4 molecule is the primary HIV-1 receptor on $CD4^+$ cells, but it also serves as a coreceptor during activation of $CD3^+$ T cells (78). Hence, the binding of gp120 (or gp160, for an anti-gp41 response) to CD4 on T cells or macrophages might assist in presenting it to the B-cell antigen receptor, irrespective of whether sufficient T-helper cells remain (54). The capture and processing of gp120 via CD4 on any antigen-presenting cell (e.g., dendritic cell or macrophage) might also increase the efficiency of presentation of gp120 to whatever residual $CD4^+$ T-helper cells remain functional, effectively amplifying T-cell help and permitting anti-gp120 production when T-cell help is insufficient to sustain an anti-Gag response.

(ii) Whereas p24 has an internal location, gp120 is presented on the surface of virions or infected cells in the form of multimeric complexes. This might permit gp120 presentation to B cells in a way unavailable to p24 (e.g., as a regular array of surface antigens). The immunogenicity of particulate antigens is relatively independent of T-cell help. Furthermore, it was recently found that, unexpectedly, a sustained, T-independent, IgG-directed antibody response to polyomavirus can completely protect T-cell-deficient mice from this lethal virus infection (88). The polyomavirus capsid protein has a regular periodicity, which lends itself to cross-linking of the B-cell antigen receptor and the evolution of a T-independent response (45, 88).

(iii) gp120 resembles few other antigens that the immune system has previously encountered. Unusually, over half its weight is carbohydrate (29, 55); the envelope glycoproteins of the oncoretroviruses have only about 10 to 20% of the gp120 carbohydrate content (24, 30). Thus, it may be that the anti-gp120 response is atypical because of the atypical nature of gp120 itself. gp41 is also glycosylated, and the anti-gp41 response is sustained as disease progresses. In contrast, most Gag antigens are not glycosylated (although minor forms of glycosylated Gag are known). New mechanisms of antigen presentation might be relevant. Non-$CD4^+$ helper cells such as the double-negative, CD1-restricted T cells (12, 20, 75, 83) or gamma/delta T cells (98) might preferentially help Env rather than Gag responses if glycosylated antigens have a relatively high affinity for antigen-presenting molecules like CD1 (12, 20, 75, 83). A second mechanism of non-CD4 antigen presentation could be mediated by the dendritic cell DEC-205 and MMR multilectin receptors (43, 77). Dendritic cells play a critical accessory role in antibody responses (34, 42). The DEC-205 and MMR receptors bind many glycoproteins via lectin-carbohydrate interactions; therefore, after internalization and processing, gp120-derived peptides could be very efficiently presented to residual $CD4^+$ T-helper cells. Alternatively, presentation of gp120 or intact virions directly via multilectin receptors on dendritic cells might be T independent, since expression of repeated determinants could lead to direct B-cell activation (9). The nonglycosylated p24 and p17 proteins would not have access to this antigen presentation mechanism.

The notion of glycosylated antigens being in a separate antigen presentation pool from nonglycosylated ones has implications for the development of neutralizing antibodies. HIV-1-positive serum rarely neutralizes contemporaneous HIV-1 isolates (2, 7, 8, 63, 91, 92, 96), exemplified by the finding that the neutralizing antibody response lags behind the emergence of gp120 variants by about 6 months (59). If gp120 (or virus) trapped on follicular dendritic cells in lymphoid tissue (34), and only slowly exchanging with plasma virus, is the antigen that generates neutralizing antibodies, then it is inevitable that this response will be mostly directed against a viral strain that disappeared from the plasma tens of HIV-1 generations ago, for there are 150 such generations per year (74). The emergence of neutralizing antibody-resistant variants in plasma may have little to do with escape from selection pressure but may be the inevitable consequence of a viral replication rate so rapid that it is drastically out of kinetic equilibrium with the antigen presentation machinery of the humoral immune system.

Quantitative aspects of antibody production bear consideration. The frequency of HIV-1-specific B cells in the blood has been variously estimated as 1:50,000 (anti-p24-specific) peripheral blood mononuclear cells (PBMC) (89), between 1:100 and 1:40,000 total B cells (4, 5); between 1:1,000 and 1:100,000 (anti-gp160-specific) PBMC (80), 1:250 peripheral blood lymphocytes (72), and 1:1,000 peripheral blood lymphocytes (82). Of total IgG-secreting B cells from clinically unselected patients, 20 to 40% were estimated as HIV-1 specific (4, 5, 82). These values can be related to estimates of IgG production rates of 500 ng/10^6 HIV-1-positive PBMC/day, equal to 10^5 molecules/PBMC/h (61). As individual B cells secrete 5×10^7 Ig molecules/h in vitro (16), about 1 in 500 of these PBMC is an HIV-1-specific B cell. We estimate that anti-gp120 increases at about 1 μg/ml/day during seroconversion, which is 5 mg of IgG/day or 10^{15} molecules/h. If in vivo and in vitro situations can be compared, about 2×10^7 B cells secrete anti-gp120 in our seroconvertors, which corresponds to about 1:100 of total circulating B cells or 1:500 PBMC, in good agreement with the estimates presented above.

Such calculations reveal the chronic stimulation of antibody production caused by HIV-1 infection and the inefficiency with which the humoral immune response controls HIV-1 replication. Only about 20 B cells are needed to generate a protective antibody concentration of 1 μg/ml during the memory phase of vesicular stomatitis virus infection of mice (10). Yet during chronic HIV-1 infection of humans, 2×10^7 B cells may secrete anti-gp120 to attain plasma concentrations in excess of 1 mg/ml. Even allowing for the 1,000-fold-greater plasma volume in humans compared to mice (3 ml compared to 5 liters), there is still a 1,000-fold excess of B cells chronically secreting antibod-

ies during HIV-1 infection compared to what is sufficient to protect a mouse from a lethal viral infection. The number of B cells active in the vesicular stomatitis virus-infected mouse during the acute phase of the immune response is greater than during the memory phase, yielding a plasma IgG concentration of ca. 500 μg/ml (10). Perhaps HIV-1 infection stimulates permanently an acute-phase type of antibody response, leading to sustained secretion of antibodies to HIV-1. The B-cell response to HIV-1 infection does not have the characteristics of a memory response (1, 34, 58). This massive anti-gp120 production may be necessary to have even a minimal effect on neutralization-resistant primary viruses (66, 67); even rare reagents with potent neutralizing activity against primary viruses protect SCID-Hu mice from infection only when present at plasma concentrations of >100 μg/ml (31).

These factors provide a sobering perspective on developing vaccines based on neutralizing antibody induction. Thus, it is urgent to discover how best to bring forth the full immunogenic potential of gp120. The antibody response to gp120 subunit vaccines is transient (15, 33, 57); peak anti-gp120 titers after boosting do sometimes approach titers found in most HIV-1-infected people, but the vaccine response decays exponentially with a half-life of 30 to 60 days. The contrast between the anti-gp120 responses to vaccination and those in naturally infected LTNP is striking. The latter sustain for many years high anti-gp120 titers induced by undetectable levels of circulating gp120. Viral load in many LTNP is below 500 virions per ml (19, 62), which at <200 gp120 molecules per virion corresponds to $<2.5 \times 10^{-14}$ g of virion-associated gp120 per ml (about 10^{-16} M). This equates to less than 100 pg of total circulating plasma gp120 antigen. In contrast, vaccinees receive up to 500 μg of gp120 per immunization (15, 33, 57), about 10^7-fold more than circulates in the plasma of LTNP. Of course, the lymphoid tissues of LTNP also harbor virions (25, 26, 73); a typical lymph node viral load of 10^8 copies of viral RNA per g of tissue (36) corresponds to around 100 ng of gp120. It is no surprise that natural HIV-1 infection induces a better antibody response than gp120 vaccination. But it can be inferred that gp120 injection intramuscularly is not the most efficient way to present it to the immune system. Thus, it would seem desirable to gain more understanding of the natural mechanisms of gp120 presentation, to enable better immunization strategies to be devised.

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