Correlation between Humoral Responses to Human Immunodeficiency Virus Type 1 Envelope and Disease Progression in Early-Stage Infection


Human immunodeficiency virus (HIV)-1–infected rapid and slow progressors showed differential humoral responses against HIV envelope peptides and proteins early in infection. Sera from slow progressors reacted more strongly with short envelope peptides modeling gp160(1-4-11), predominantly in gp41. Reactivity to six peptides (in constant regions C3, C4, and C5 of gp120 and in gp41) correlated with slower progression. In a novel association, reactivity to three peptides (in constant regions C1 and C3 and variable region V3 of gp120) correlated with faster progression. Envelope peptide reactivity correlated with subsequent course of disease progression as strongly as did reactivity to gag p24. Patients heterozygous for 32-bp deletions in the CCR5 coreceptor reacted more frequently to an epitope in gp41. Rapid progressors had greater gp120 native-to-denatured binding ratios than did slow progressors. While antibody-dependent cellular cytotoxicity against gp120 did not strongly differentiate the groups, slow progressors showed a broader neutralization pattern against 5 primary virus isolates.

One of the major problems facing human immunodeficiency virus (HIV) vaccine development is lack of understanding of the correlates of immunity for this virus [1, 2]. Infection with HIV leads to eventual development of AIDS, and death, in the vast majority of untreated persons [3, 4]. Survival after infection with HIV-1 can be modeled by a normal distribution, with median time to clinical symptoms of ∼10 years [3, 5]. At least three distinguishable patterns have been determined for persons with unusually long survival times. The first includes those infected with less virulent viruses, including persistently macrophage-tropic isolates [6], and attenuated viruses [7, 8], such as viruses with nonfunctional nef genes [7, 9, 10]. The second pattern includes those with specific host factors. For example, subjects with CCR5 coreceptor gene defects or certain HLA profiles show delayed disease progression rates [11–14]. The final group includes all other cases, including those at least partially attributable to specific immunologic control of viral pathogenicity. Envelope-directed humoral responses in this group are the subject of this study.

Previous workers have correlated humoral responses with rate of progression in HIV-1–infected cohorts. Higher anti-p24 antibody levels correlate with slower progression [15–17]. Although higher anti-gp120 antibody levels have been correlated with rapid progression [16], this finding is controversial [18]. Stronger reactivity to the C-terminus of gp120 correlates with slower progression [19]. In functional antibody assays, studies of antibody-dependent cellular cytotoxicity (ADCC) directed against envelope (gp120) have yielded contradictory data. Development and maintenance of higher ADCC correlated with slower progression in adults [20, 21] and children [22, 23]; however, no such correlation was found in other studies [18, 24]. Studies of neutralization of virus by serum antibodies are also in disagreement. Stronger neutralization against laboratory isolates correlated with improved disease outcome in some studies [8, 25] but not in all [18]. Slower progressors are able to neutralize primary isolates more frequently than are faster progressors [25–27].

We have measured the entire set of humoral correlates of immunity described above in a single progression cohort. To measure anti-peptide responses, we used a modified pepscan assay [28], which has proven useful for measuring anti-HIV
envelope responses in previous studies [29–31]. The significant predictive epitopes identified were then compared in 2 unrelated cohorts for their independent predictive value. Sera were compared from infected persons only before clinical progression had occurred, to emphasize early responses rather than those that develop or change during the progression of infection.

Methods

Subjects and Specimens Tested

Three progression panels were used.

Walter Reed cohort. The majority of experiments were carried out on a panel that included early-stage (Walter Reed stage 1 or 2 with CD4 cell counts >400/mm³) HIV-infected volunteers enrolled in a vaccine therapy trial (n = 140) (table 1) [2]. Only sera obtained before any immunization were used in this study. Because there was no indication of clinical efficacy in the trial, patients were divided into progression groups without regard to trial arm [2]. Rapid progressors (RP) were defined by death from HIV-related diseases within 60 months from study entry (n = 13; mean time to death, 42 months). Slow progressors (SP) were defined on the basis of the following criteria: lack of progression in Walter Reed disease stage past stage 2, CD4 cell counts continuously >400/mm³, serum RNA virus load <1000 copies/mL continuously throughout follow-up, and lack of antiretroviral treatment (n = 13; mean follow-up 56 months). All patient charts were independently reviewed by physicians before final staging. In the progression cohort, 2 RP and 3 SP were heterozygous for the recently described 32-bp deletion in the CCR5 coreceptor molecule (CCR5 +/−). These volunteers were excluded from the initial analysis and then considered separately. Date of seroconversion was calculated, when possible, as the midpoint between last known seronegative date and first measured seropositive date; a known seronegative date was available for 6 RP (1 CCR5 +/−) and 6 SP (2 CCR5 +/−).

Stockholm cohort. Sera were obtained from a similar trial carried out by the Swedish Institutes for Infectious Disease Control and the Karolinska Institute, Stockholm (n = 40) [32]. Patients have participated since 1991, and all were receiving vaccine. At the time the sera were taken, all had CD4 cell counts >400/mm³. In the RP group (n = 11, including 1 CCR5 +/−), CD4 cell counts declined with slopes of greater than or equal to −7/mm³/month over the next 5 years, while in the SP group (n = 11, 3 CCR5 +/−), CD4 cell counts were unchanged or increased over the same time period.

San Francisco Men’s Health Study (SFMHS) cohort. Sera were obtained from a population-based cohort of seropositive, single men recruited since 1984 in San Francisco [17]. Volunteers had been monitored semiannually for up to 114 months as of the time sera were taken for this study. Volunteers that reached the Centers for Disease Control and Prevention definition of AIDS in ≤72 months were classified as RP (9 randomly selected sera were used from this group, including 2 CCR5 +/−); volunteers that had not progressed to AIDS during the entire follow-up were classified as SP (10 sera were taken from this group, 2 CCR5 +/−).

Table 1. Characteristics of the Walter Reed progression cohort.

<table>
<thead>
<tr>
<th>Status</th>
<th>Rapid progressors</th>
<th>Slow progressors</th>
<th>Significancea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numberb</td>
<td>11</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Initial Walter Reed stage</td>
<td>2</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Final Walter Reed stage</td>
<td>Deathc</td>
<td>2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Time since seroconversion (months)</td>
<td>31.5d</td>
<td>44.5d</td>
<td>NS</td>
</tr>
<tr>
<td>Initial CD4 cell count/mm³</td>
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<td>948</td>
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</tr>
<tr>
<td>Final CD4 cell count/mm³</td>
<td>42</td>
<td>939</td>
<td>&lt;.001</td>
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<tr>
<td>CD4 cell count slope (cells/mm³/month)</td>
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<td>−0.7</td>
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<tr>
<td>Initial virus load (log RNA copies/mL)</td>
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<td>2.4</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Final virus load (log RNA copies/mL)</td>
<td>5.3</td>
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<td>&lt;.001</td>
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<tr>
<td>Virus load slope (log RNA copies/mL/month)</td>
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<td>−0.003</td>
<td>&lt;.001</td>
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<tr>
<td>Initial anti-p24 antibody (mOD/min)</td>
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<td>58</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

a By Mann-Whitney nonparametric test (NS, not significant).
b Total n = 26; CCR5 +/− subjects (2 rapid, 3 slow progressors) were removed from this analysis; no significant changes were observed when CCR5 +/− subjects were included.
c For calculation purposes, Walter Reed stage 6 was used.
d n = 5 rapid and 4 slow progressors.

Linear Mapping Assays

Peptides were 12 mers overlapping by 8 aa defining the entire gp160 sequence of HIV-1NL4-3 (212 peptides) [33]. They were synthesized on the heads of synthetic pins with cleavable di-ketopiperazine linkages (Chiron Mimotopes, San Diego) using Fmoc chemistry [31, 34, 35]. Peptides were covalently linked to biotin at the N-terminus by a short peptide linker (Ser-Gly-Ser-Gly). Peptides were freed from the pins by recommended procedures and stored at −80°C until used. Peptides are denoted by N-terminal amino acid. The synthesis was checked by amino acid analysis on control peptides and peptides of interest. In addition, the ability of each peptide to block binding of biotinylated horseradish peroxidase to streptavidin plates was used as proof of completion of the synthesis. Previous results using this method have typically yielded short peptides of >90% purity [36, 37]; we observed comparable results. Peptides were used without further purification as antigens in an ELISA [34]. Briefly, plates were coated with streptavidin (0.25 µg/well) overnight and then peptides (0.1 µg/well) for 1 h. Sera were diluted 1:200 in 5% nonfat dry milk in PBS and incubated with peptide antigens for 2 h at room temperature. After washing, anti-human secondary antibody was added at 1:1000 for 1 h. Appropriate substrate was added and plates were read at 30 min and 2 h. All assays were carried out in duplicate. Data were converted to reactivity in standard deviations (σ) from the
median reactivity to all peptides; peptides with values above 5 σ were considered significantly reactive [38].

ELISAs

Antigens used included p24 (MicroGeneSys, Meriden, CT), 1 μg/mL, and V3 loop peptides: V3(LAI), TRPNNTRKSI1-RGPGRAFVTIGKNMQ; V3(MN), TRPYNKRKRI-HIGPGRAFTKNIITQ; and V3(CM237), CTRPNN-TNTRKSIHLGPGKAWTTGGIQGDHDAC (Synthecell, Gaithersburg, MD), 20 μg/mL, and V3(429) peptides were synthesized using Fmoc chemistry and standard solid-phase techniques (Excel automated synthesizer; Waters, Milford, MA). Proof of structure was obtained by high-pressure liquid chromatography, amino acid analysis, and N-terminal sequencing. Individual peptides from the linear mapping synthesis were used in this format by coating plates with streptavidin and coating multiple wells with one peptide. Antigen was diluted to its optimized concentration in carbonate buffer, overnight. Antigen was removed, and sera diluted at optimized concentration in PBS containing 5% nonfat dried milk were allowed to bind for 2 h at room temperature. After washing in PBS containing 0.1% Tween 20, anti-human secondary antibody was added at 1:1000 in 5% milk for 1 h. Appropriate substrate was added, and changes in absorbance were read kinetically at 405 nm over 6 min; results are given as signal in milli-optical density units (mOD)/min at the optimized dilution. Background absorbances (serum reactivity against plates kinetically at 405 nm over 6 min; results are given as signal in resonance units, RU) after normalizing for any differences in the rgp120 and V3(NH-Z) peptides were synthesized using Fmoc chemistry and standard solid-phase techniques (Excel automated synthesizer; Waters, Milford, MA). Proof of structure was obtained by high-pressure liquid chromatography, amino acid analysis, and N-terminal sequencing. Individual peptides from the linear mapping synthesis were used in this format by coating plates with streptavidin and coating multiple wells with one peptide. Antigen was diluted to its optimized concentration in carbonate buffer, overnight. Antigen was removed, and sera diluted at optimized concentration in PBS containing 5% nonfat dried milk were allowed to bind for 2 h at room temperature. After washing in PBS containing 0.1% Tween 20, anti-human secondary antibody was added at 1:1000 in 5% milk for 1 h. Appropriate substrate was added, and changes in absorbance were read kinetically at 405 nm over 6 min; results are given as signal in milli-optical density units (mOD)/min at the optimized dilution. Background absorbances (serum reactivity against plates without peptide) were subtracted. All assays were carried out in triplicate.

Competition ELISA

D7324 (Aalto BioReagents, Dublin) is a sheep polyclonal antiserum to the C-terminus of gp120; 1359 [39] is a murine monoclonal antibody to a linear epitope in the C1 domain of gp120. Purified recombinant monomeric gp120, derived from HIV-1, was expressed in CHO cells [40], was a gift from P. Maddon and G. Allaway (Progenics Pharmaceuticals, Tarrytown, NY). Recombinant gp120 was captured on a 96-well microtiter plate via absorbed 135/9. Serum responses to the D7324 were considered signiﬁcantly reactive [38]. All values were corrected for background absorbance without sera and biotin-labeled D7324. Absorption from wells with sera but without D7324 was never greater than background and so was excluded from the analysis. Binding curves derived from antibody titrations were plotted, and the midpoint titers (50% inhibition of biotin-D7324 binding) were determined graphically.

Surface Plasmon Resonance

Binding interactions between ligand (gp120 [Genentech, South San Francisco] or gp160 [Advanced Bioscience Laboratories, Kensington, MD]) covalently linked to a biosensor matrix and ligate (antibodies in solution) were measured using surface plasmon resonance (BLAcore; Pharmacia Biosensor, Piscataway, NJ) [41]. Appropriate matrices were prepared by injecting across the activated matrix 30 μL of the following: recombinant (r) gp120 (20 μg/mL in sodium acetate buffer, NaAc, pH 4.5); rcmgp120 (20 μg/mL in NaAc, pH 5.0); reduced and carboxymethylated (rcm) gp120IIIB (70 μg/mL in NaAc, pH 4.0); rcmgp120MN (35 μg/mL in NaAc, pH 4.0); gp160 451 (20 μg/mL in NaAc, pH 5.0); or gp160 451 (20 μg/mL in MES, pH 6). Matrices were blocked with 1 M ethanolamine. Next, 30-μL aliquots of sera and control monoclonal antibodies diluted in running buffer (10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 0.05% BSA) were run through the matrices at 5 μL/min. The native-to-denatured gp120 antibody-binding ratios were determined by dividing the amount of serum binding to rgp120 binding (in resonance units, RU) by the amount of serum binding to rcmgp120 binding (in RU) after normalizing for any differences in the rgp120 and rcmgp120 matrix concentrations. Control monoclonal antibodies previously shown to bind speciﬁcally to denatured gp120 (1C1), to native gp120 (P43110), or to both native and denatured gp120 (V3) were used as controls. Matrices were regenerated using 60 mM H3PO4 (rpg120, rcmgp120) or 10% formic acid (gp160) before injection of the next sample.

ADCC

CEM.NK target cells (AIDS Research and Reference Program, NIH, Bethesda, MD) were labeled with 300 μCi of sodium 51Cr per 3 × 106 cells for 1 h at 37°C. Excess 51Cr label was washed off, and the cells were incubated with 10 μg of recombinant gp120 (Intralcell, Cambridge, MA) per 106 cells. Target cells were plated out at 5 × 103 cells/well and incubated for 20 min with 50 μL of 10-fold serial dilutions (from 104 to 106) of heat-inactivated sera from HIV-infected patients. Blood from normal donors was used as the source of effector cells. Peripheral blood mononuclear cells (PBMC), obtained by density gradient centrifugation from a single donor, were used to test ADCC activity of all the sera; cryopreserved PBMC from this donor were thawed and incubated overnight and effector PBMC added to the targets at an effector-to-target...
ratio of 100:1. The effectors, serum, and target cells were incubated together for 6 h. $^{51}$Cr release was measured by gamma scintillation counting (Cobra, Packard, CT), and percentage of specific lysis was calculated as $100 \times \frac{[\text{mean test cpm} - \text{mean cpm}]}{[\text{mean max cpm} - \text{mean cpm}]}$, where test cpm = counts per minute released by the CEM.NKr target cells in the presence of effector cells, spon cpm = cpm released by the CEM.NKr target cells in the absence of any effector cells, and max cpm = cpm released by the target cells in the presence of S DS. The percentage of release from the CEM.NKr in the presence of normal human serum did not exceed 5%.

Neutralization Assays

Virus isolation was done by cocultivation of ficoll-separated PBMC from HIV-1–infected subjects with phytohemagglutinin-stimulated uninfected donor PBMC. All viruses tested were from subtype B and were non–syncytium-inducing in MT2 cells, replicating preferentially in HOS.CD4 cells expressing the CCR5 coreceptor [14]. Viral growth kinetics and TCID$_{50}$ were determined within the assay format [42]. The same donor cryopreserved PBMC were used in the titration and neutralization assays. Sera were heat-inactivated (56°C for 45 min) before use and assayed at a single dilution of 1:5. Twenty-five milliliters of test serum per well was aliquoted in triplicate wells of a 96-well microtiter plate. Sextuplicate wells with pooled, heat-inactivated normal human serum served as the control for baseline viral growth. The cells were washed four times in a 96-well culture box that holds 500 µL/well, yielding a final serum dilution factor of >1:100,000. As we have previously shown, this dilution is sufficient to remove interfering levels of anti-p24 antibody [43]. Twenty-five milliliters of virus stock, 100 TCID$_{50}$, was added to each well. After 30 min at 37°C, $2 \times 10^7$ phytohemagglutinin-stimulated PBMC were added and incubated overnight at 37°C. Cells were then washed extensively and transferred to a 96-well microtiter plate with culture media containing interleukin-2. Inhibition of PBMC infection was quantitated by measurement of p24 in cell supernatants during the early viral growth phase (day 4 or 5 for the viruses in this study). For each serum, the result was reported as the fold decrease of p24 antigen compared with control (mean p24 antigen of control wells/mean p24 antigen in test sera wells).

Statistical Methods

Unless stated otherwise, all comparisons between groups were made with the Mann-Whitney nonparametric test, using Statview 4.01 (Abacus, Berkeley, CA).

Results

Patient staging. Clinically, RP and SP from the Walter Reed cohort were indistinguishable at the time sera were drawn (table 1). All patients were early-stage (Walter Reed 1 or 2), with CD4 cell counts >400, and had never exhibited symptoms beyond local lymphadenopathy. Time since seroconversion was not significantly different ($P > .8$) between the 2 groups (using the subset of subjects for whom the seroconversion date could be estimated). By the end of the trial (up to 5 years later) the 2 groups had diverged significantly in stage. The RP had all died, whereas the median SP stage had not changed. Even though the 2 groups were clinically indistinguishable at the early time point, they differed by several laboratory parameters then, including CD4 cell count, viral RNA load, and p24 antibody titer. Table 1 highlights the differences used to choose the SP retrospectively, including CD4 cell count slope and viral RNA loads.

Linear epitope mapping. The antibody responses of the 2 groups to overlapping peptides modeling gp160 were compared (figure 1). Consistent with previous results [29–31], at least 50% of volunteers from both groups were reactive with peptides from several regions. These included peptides 301 (71% overall, median reactivity 37 $\sigma$) and 305 (86%, 92 $\sigma$) in the V3 loop, peptide 497 (81%, 55 $\sigma$) near the C-terminus of gp120, peptides 585–597 (100% reactivity with each, all with reactivities >100 $\sigma$) from the immunodominant region in gp41, peptide 657 (91%, 17 $\sigma$) in the extracellular part of gp41, and peptide 793 (67%, 47 $\sigma$) from the cytoplasmic tail of gp41. The cluster near aa 600 in gp41 dominated the reactivity profile, as previously described [44].

The breadth of activity was similar; RP and SP were reactive with similar numbers of envelope peptides (median, 33 reactive peptides each of 212). In magnitude, however, SP had significantly stronger total reactivity to the peptide set when all individual reactivities were summed. The difference between the total peptide reactivity of the 2 cohorts was significant in gp41 ($P = .01$) and overall gp160 ($P = .04$) but not gp120 (figure 2A). Much of the reactivity difference between the cohorts was outside the immunodominant region of gp41 ($P = .008$ for gp41 or .009 for gp160 when these peptides were excluded [figure 2B]).

The difference in magnitude of response was reflected in activity to individual peptide epitopes; those with the largest differential reactivity are summarized in table 2. SP reacted more strongly with 8 peptides; 3 of the differences were significant ($P < .05$), 3 more were significant if CCR5 +/− subjects were included, and differences between the remaining two peptides (653 and 657 in gp41) did not reach significance. Three peptides were significantly more reactive with RP sera than SP Reactivities of sera from CCR5 +/− volunteers were also investigated separately (table 2). The largest differences in reactivity between CCR5 +/− and CCR5 +/+ subjects were observed to three peptides (489, 793, 841). The difference was significant only for peptide 793 ($P = .03$).

The mapping results suggested that reactivity to individual peptides might be useful in distinguishing RP and SP. The pep-
tides of interest were used in an ELISA to test their predictive value. Six of the peptides were selected to differentiate between RP and SP. Of these, SP reacted more strongly with three (“slow peptides”: 501, 581, and 653) and RP reacted more strongly with the other three (“rapid peptides”: 101, 301, and 353). Peptide 497 was excluded because of its overlap with peptide 501. Reactivity to peptide 597 was very strong, requiring different assay conditions, so it was not included in further predictive tests. Reactivity to peptides 341 and 413 was too weak to consistently differentiate between the 2 groups in this assay format. Peptide 653 was included because it contains a known broadly neutralizing epitope [45, 46].

Although individual peptides distinguish between the 2 cohorts, no single peptide reactivity served to differentiate all of the individual members of either group from the other. An algorithm was developed to use total reactivity to these peptides to better distinguish between the groups. A score was obtained for each serum by comparing four types of reactivity. The first, \( S_n \), was the number of slow peptides (501, 581, and 653) the serum reacted with (possible total of 0–3). Reactivities for rapid peptides were established in the same way and were designated \( R_n \) for qualitative and quantitative response, respectively. The total peptide reactivity was scored by the following equation: reactivity score = \( (S_n + S_p) - (R_n + R_p) \). A serum strongly reactive to peptides 501, 581, and 653 but completely negative to peptides 101, 301, and 353 could receive a maximum score of 6. The scores achieved by the sera in the Walter Reed cohort are summarized in figure 3. The median value for SP was 1.6, and 9 of 10 scored above 0, whereas the median value for RP was −0.6, and all 11 scored below 0. This reactivity score strongly differentiated between the groups (\( P < .001 \)).

**Comparison to alternate cohorts.** This model was then tested for predictive value in 2 cohorts unrelated to the one used to develop it. Sera were obtained from the Stockholm gp160 trial and SFMHS rapid/slow cohort (see Methods). Antibody levels to the six envelope peptides described above were measured (table 3). This test differentiated between the RP and SP of both cohorts with similar confidence (\( P < .05 \) for each comparison). When the results obtained from all 3 cohorts were combined, the tests were highly significant, with median envelope peptide reactivity score differentiating between RP and SP (\( P < .001 \)). For comparison, anti-p24 reactivity differenti-

![Figure 1. Summary of reactivity by peptide mapping, Walter Reed cohort: Data are % of reactivity (number reactive/total) of rapid progressors (○; \( n = 11 \)) and slow progressors (■; \( n = 10 \)) to each of overlapping peptides representing gp160 NL4-3. Lengths of connecting lines show differences in reactivity between rapid and slow progressors.](https://academic.oup.com/jid/article-abstract/178/5/1306/810369/136b710a98?download=true)
ated between the RP and SP in the SFMHS cohort ($P = .037$) but did not reach significance in the Stockholm cohort ($P = .091$). Combining the anti-p24 antibody results obtained from all 3 cohorts differentiated between RP and SP with $P < .001$.

Antibody levels to V3 and the gp41 immunodominant region. That reactivity to an epitope in the V3 loop, a known neutralizing region, correlated with RP was somewhat surprising. Median reactivity was higher in the RP group than in the SP group to three other clade B V3 peptides (Bcons: RP = 47 mOD/min, SP = 33 mOD/min; MN: RP = 49 mOD/min, SP = 41 mOD/min; TN237: RP = 36 mOD/min, SP = 9 mOD/min), but none of the differences were significant ($P > .5$ for each). Of interest, reactivity to the full-length NL4-3 peptide was markedly reduced compared with that to the 12-mer peptides that modeled it (NL4-3: RP = 0.31 mOD/min, SP = 0.16 mOD/min, $P = .5$). Sera were unreactive with the HIV-2 (NIH-Z) sequence.

Peptide 597 contains all of the amino acids in the small disulfide-linked immunodominant loop in gp41, except for the N-terminal cysteine. This peptide was significantly more reactive with SP sera (table 2). When reactivity to all peptides in the immunodominant cluster (peptides 577–605) was compared, total responses of 674 $\delta$ (RP) and 839 $\delta$ (SP) were obtained ($P = .17$). Reactivity against peptide 593 (IWGC\textsuperscript{SGK}L\textsuperscript{I}C\textsuperscript{T}T), which contains this loop entirely, was also tested using the 3 combined cohorts. The ELISA was done with the peptide in both the oxidized and reduced forms; neither test showed significant correlation with progression. A typical experiment yielded median reactivities of 58 mOD/min at 1:10,000 dilution for RP compared with 79 mOD/min for SP ($P = .23$).

Reactivity of sera from CCR5 +/− subjects. The results from the Walter Reed cohort suggested that there was differential peptide reactivity in sera from CCR5 +/− and CCR5 +/+ subjects (table 2). Reactivity was compared using the sera from all 3 cohorts ($n = 13$ CCR5 +/− and 54 CCR5 +/+). Different reactivities to peptides 489 and 841 were no longer observed when all cohorts were combined. However, sera from CCR5 +/− subjects reacted more strongly with peptide 793 (median, 21 mOD/min at 1:200 dilution) than did sera from CCR5 +/+ patients (median, 2.5 mOD/min) ($P = .005$).

Competition ELISA. In the Walter Reed cohort, 6 of the SP sera were capable of competing for binding of monoclonal antibody D7324 to captured gp120 (median titer, 1.59), compared with only 2 of the RP sera competing (median titer, 0.22) ($P < .03$). This is consistent with the binding results observed with peptide 501. There was no significant difference observed when blocking of CD4 was measured (data not shown).

Antibodies to full-length envelope proteins. Reactivity against full-length envelope proteins was measured by surface plasmon resonance (figure 4A). Although the median reactivity of the SP group was lower to oligomeric gp160 from 2 viruses (gp160\textsubscript{CDC451} and gp160\textsubscript{llb}) than that observed in the RP group,
Table 2. Reactivity of specific peptides with sera from Walter Reed progression cohort.

<table>
<thead>
<tr>
<th>Purpose, peptide</th>
<th>Sequence</th>
<th>Rapid progressors</th>
<th>Slow progressors</th>
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<th>Including CCR5+/−</th>
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</table>

* Median reactivity of sera from each group by pepscan ELISA. Bolded values are higher than in companion group.

b Calculated by Mann-Whitney nonparametric test.

Figure 3. Difference in peptide reactivity between slow (shaded box) and rapid (open box) progressors, Walter Reed cohort. Data are total reactivity score to envelope peptides of interest. Box plots show first decile, first quartile, median, third quartile, and ninth decile; values above boxes are medians. Individual value points are also shown.

ADCC. ADCC activity against gp120 was compared between the 2 groups. RP had stronger ADCC directed against envelope than did SP at all dilutions tested. The best dynamic range was obtained at a serum dilution of 10^−5 (figure 4B). When activities of the sera from the CCR5+/− volunteers were included, however, the difference was emphasized (RP = 8.4, SP = 5.4; P = .013). When the 3 cohorts were combined (excluding CCR5+/− subjects), the ratio was 7.8 for RP and 5.5 for SP (P = .005), indicating a preference by SP for binding linear epitopes (figure 4C). When the CCR5+/− data were included, this preference was even stronger (RP = 8.1, SP = 5.2; P = .002). Reactivities to rgp120_MN and rcm120_MN were barely detectable, so a similar ratio could not be calculated with this genotype (data not shown).

Neutralization assays. Ability of the sera to neutralize unrelated primary clade B virus isolates was measured. RP and SP had significantly different neutralization activity against only 1 of these, US1 (RP: median, 4-fold reduction; SP: median, 37-fold; P = .02). Comparing all SP sera to all RP sera, using geometric mean neutralization of all five viruses, showed a trend toward greater reactivity among SP sera (RP: 7.4-fold reduction; SP: 21-fold; P = .06) (figure 5B). When neutralization was defined as a 10-fold reduction in infectivity, 25 of the 50 SP serum-virus combinations were neutralizing (n = 10 sera), whereas the 11 RP sera neutralized only 12 of 55 possible combinations. This difference was highly significant (χ² = 9.115, P = .002).
Table 3. Ability to distinguish rapid from slow progressors by using reactivity to gp160 peptides and to p24; comparison between 3 cohorts.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>n</th>
<th>ELISA reactivity to gp160 peptides</th>
<th>ELISA reactivity to p24</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rapid progressors</td>
<td>Slow progressors</td>
</tr>
<tr>
<td>Walter Reed</td>
<td>11</td>
<td>0.80</td>
<td>2.10</td>
</tr>
<tr>
<td>Stockholm</td>
<td>10</td>
<td>0.63</td>
<td>1.68</td>
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<tr>
<td>San Francisco Men's Health Study</td>
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<td>0.00</td>
<td>2.24</td>
</tr>
<tr>
<td>All combined</td>
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<td>0.00</td>
<td>2.16</td>
</tr>
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</table>

* By Mann-Whitney nonparametric analysis.

Discussion

This comprehensive analysis of antibodies directed against HIV-1 envelope yielded information from specifically predictive linear epitopes to functional antibody analysis. Peptide mapping yielded new data on the relationship between progression and antibody reactivity to linear epitopes on the envelope. The linear mapping assay (pepscan) was more sensitive for detection of peptide reactivity than a comparable ELISA format because the background absorbance of a given serum could be determined more accurately in the former [38]. Overall reactivity to linear epitopes in gp160 correlates with subsequent slower progression. This difference, evident throughout gp41, is observed especially outside the immunodominant cluster near aa 600. Total peptide reactivity identified this difference better than did reactivity to whole linearized envelope, in which reactivity to this cluster may obscure differences. The ratio of binding to native compared with binding to denatured envelope also showed greater binding of SP to linearized epitopes.

Reactivity to several individual epitopes was found to correlate with slower progression. Associations between reactivity to individual envelope peptides and disease stage have been reported previously. Peptides from the C-terminus of gp120, around peptide 501, inhibit serum neutralization of T cell line–adapted virus [45]. Antibodies directed against this region were neutralizing in some studies [47] but not others [48]. Reactivity to this region increases in SP and decreases in RP over time [19]. Reduction in titer against the region containing peptide 581 correlated with disease stage in some studies [49, 50] but not others [51–53]. Differences in reactivity to the immunodominant loop in gp41 have been correlated with progression.

Figure 4. Anti-envelope antibody levels by BIAcore in rapid progressors (open boxes) and slow progressors (shaded boxes). A, Reactivity to full-length HIV-1 envelope proteins, including gp160, gp160, recombinant (r) gp120, and reduced and carboxymethylated (rcm) gp120. B, Ratio of reactivity to rgp120/reactivity to rcmgp120, Walter Reed cohort. C, Ratio of reactivity to rgp120/reactivity to rcmgp120, 3 cohorts combined. Box plots show first decile, first quartile, median, third quartile, and ninth decile; values above boxes are medians.
Cell counts had fallen significantly (median, 80/mm³) in one cohort followed longitudinally [58], and a human monoclonal antibody to this epitope is neutralizing [54]. Peptide 653 contains a broadly neutralizing epitope [45, 46], and reduction in titer against this region correlates with stage of disease [49, 50].

We show, for the first time, an association between antibody reactivities to specific epitopes and faster progression. Perhaps these antibodies are blocking binding of functional antibodies and thus contributing to disease progression. Alternatively, RP may present envelope epitopes differently than do SP. The majority of monoclonal antibodies developed against the region in C1 containing peptide 101 and the region in C3 containing peptide 353 bind to denatured antigen better than to native protein [55]. Peptide 301 is part of the V3 loop, probably the most studied region of the HIV virion. Antibodies against this region neutralize both laboratory isolates and, at high concentrations, primary virus isolates (generally with isolate specificity) [29, 47, 56]. Previously, increased reactivity to V3 loop peptides was correlated with slower progression for two genotypes, BH10 [49] and MN [57]. In these studies, sera from RP were drawn later in disease (12–14 months before death); CD4 cell counts had fallen significantly (median, 80/mm³) in one study [49] and were not reported in the other [57]. One explanation that reconciles these observations with the current findings is that anti-V3 antibodies rise early in infection, especially in RP, but decrease as clinical disease develops. This explanation is consistent with results reported using a seroconversion cohort followed longitudinally [58].

CCR5 +/- subjects were excluded in the first analysis of these data, since the CCR5 genotype is independently associated with disease progression [11–14]. For the most part, including responses from CCR5 +/- subjects did not significantly change results; minor changes in statistical conclusions could simply have resulted from changes in number of subjects evaluated. The major exception was in reactivity to peptide 793, in which the CCR5 +/- subjects acted more as a defined cohort, with significantly increased responses compared with CCR5 +/+ subjects. Antibody responses directed against this region in HIV-infected volunteers have been previously reported [59]. Functional significance has been attributed to these responses; strong maternal serum reactivity to a peptide containing this epitope, MN(791–810), correlated with lack of maternal-infant transmission in a recent study [60].

Previous studies showed that titer of antibodies to p24 is an important immunologic correlate of progression. High levels of antibodies to p24 develop early after infection but decline subsequently or never develop at all in Rp [6, 15, 16]. In comparison, linkage between response to the entire envelope and rate of progression has been controversial. Our results support a correlation between progression and reactivity to envelope peptides, as well as to p24, in 3 unrelated cohorts. The conclusions reached by examining responses to peptides are different from those obtained when only responses to gp120 or the immunodominant peptide epitope in gp41 are measured [51].

Peptide mapping showed differences in reactivity between Rp and Sp when the 2 groups were clinically indistinguishable. Although the groups could be differentiated by CD4 cell count, the initial counts were quite high in both groups (Table 1). In addition, both groups had similar antibody responses to conformationally intact, oligomeric gp140. Thus, although reactivity to envelope peptides may be simply another marker of overall immunologic health, the possibility that these reactivities are contributing to decreased rate of progression cannot be eliminated.

Two possible functions of antibodies to envelope are in directing ADCC against infected cells and in direct neutralization of virus. In this study, slow progression was not correlated with higher ADCC; instead, a trend toward the opposite was found. This is consistent with other studies [18, 24]. Two previous studies finding higher ADCC in Sp were conducted in pediatric cohorts [22, 23], and one of the remaining studies used gp120 or gp160 labeled target cells [21].

Ability to neutralize specific wild type viruses in PBMC correlated with slower progression for only 1 of 5 individual viruses tested in this study, but breadth of neutralization capability against multiple isolates did correlate with clinical outcome. Sera were collected early in infection, which may minimize neutralizing ability gained over the course of infection. Even so, the value of 50% of virus-serum combinations being neutralizing in Sp is consistent with 56% reported recently in long-term nonprogressors [26] and in the range reported in another cohort over time (37% early, 85% 6 years later, n = 6) [27].

In conclusion, these studies show that sera from Rp and Sp
can be differentiated by reactivity to a panel of envelope peptides. In this study, such reactivity proved as useful a prognostic indicator as reactivity to p24. Differences in reactivity occur early in infection, before clinical symptoms occur and before CD4 cell counts drop below 400. This information may prove useful in monitoring progression during HIV infection and perhaps help guide vaccine development.

Acknowledgments

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References

11. Dean M, Carrington M, Winkler C, et al. Genetic restriction of HIV-1 infection early in infection, before clinical symptoms occur and before CD4 cell counts drop below 400. This information may prove useful in monitoring progression during HIV infection and perhaps help guide vaccine development.

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We acknowledge technical assistance from M. Mitchell, S. Connors, G. Chang, and D. Dayhoff (H. M. Jackson Foundation) and A. Morris and A. Sanborn (SRA Technologies). We thank John Moore (Aaron Diamond AIDS Research Center) for review of the manuscript and help in designing the antibody competition experiments.

References


47. Prieto I, Hervas-Stubbs S, Garcia-Granero M, et al. Simple strategy to induce
44. Wang J, Steel S, Montagnier L, Sonigo P. Detection of antibodies to human
43. Mascola JR, Burke DS. Antigen detection in neutralization assays: high levels
42. Mascola JR, Snyder SW, Weislow OS, et al. Immunization with envelope
41. VanCott TC, Loomis LD, Redfield RR, Birx DL. Real-time biospecific in-
40. Trkola A, Dragic T, Arthos J, et al. CD4-dependent, antibody-sensitive in-
38. Loomis-Price LD, Levi M, Burnett PR, et al. Linear epitope mapping of
37. Valerio RM, Benstead M, Bray AM, Campbell RA, Maeji NJ. Synthesis of
35. Carter JM. Epitope mapping of a protein using the Geysen (PEPSCAN) method.
34. Loomis-Price LD. Linear epitope mapping by the PEPSCAN method. In: