Self Antigen Prognostic for Human Immunodeficiency Virus Disease Progression

CYNTHIA L. BRISTOW,1* HIRENKUMAR PATEL,2 AND ROLAND R. ARNOLD2

Department of Pathology and Laboratory Medicine1 and Dental Research Center,2
University of North Carolina—Chapel Hill, Chapel Hill, North Carolina 27514

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We have recently found that an extracellular protein, α1 proteinase inhibitor (α1PI; α1 antitrypsin), is required for in vitro human immunodeficiency virus (HIV) infectivity outcome. We show here in a study of HIV-seropositive patients that decreased viral load is significantly correlated with decreased circulating α1PI. In the asymptomatic category of HIV disease, 100% of patients manifest deficient levels of active α1PI, a condition known to lead to degenerative lung diseases and a dramatically reduced life span. Further, HIV-associated α1PI deficiency is correlated with circulating anti-α1PI immunoglobulin G. These results suggest that preventing HIV-associated α1PI deficiency may provide a strategic target for preventing HIV-associated pathophysiology.

In general, proteinase inhibitors are inhibitory for a single class of proteinases, often resulting in mutual inactivation. On the other hand, proteinases may inactivate many classes of inhibitors without being inactivated themselves and, in the process, may produce bioactive fragments, e.g., complement fragments. The proteinase inhibitor in serum exhibiting the greatest concentration is α1PI, and the proteinase inhibitor encompassing the broadest spectrum is α2 macroglobulin (α2M). Multiple molecular conformations of active and inactive α1PI are known to exist and to be receptor recognized. For example, active α1PI has been found to react with surface-associated human leukocyte elastase (HLE), and inactive α1PI has been found to react with the well-characterized scavenger receptor, α2M receptor–low-density-lipoprotein receptor-related protein (α2MR-LRP) (2, 21). The highly conserved α1PI uniquely inhibits all four classes of proteinases and exhibits a receptor-recognized conformation involved in signaling as well as an α2MR-LRP-recognized conformation (17). A significant body of evidence suggests that receptor recognition of the multiple forms of circulating proteinase inhibitors results in activation of discrete cellular subsets of the mononuclear phagocyte system (27).

Whereas α1PI covalently inhibits the catalytic site of HLE, it has been shown that α2M binds to the catalytic site of HLE in plasma is favored (6). When proteinases are complexed with α2M, proteolysis of low-molecular-weight peptides and cytokines can persist. The balance between available HLE activity, the inhibitor α2PI, and the substrate-restricting α2M represents a tightly regulated mechanism for discrete targeting of proteolytic activity in tissues. It has been shown that inter-α-trypsin inhibitor (Iα1) uniquely acts as a shuttle by transferring HLE to α1PI or α2M (25). It is the active site of Iα1 which is identical with the principle HIV-neutralizing determinant in the V3 loop, and it is this region of the V3 loop which has been shown to reversibly inhibit proteinases (1). This suggests the possibility that HIV envelope proteins themselves may disrupt the homoeostasis between proteinases and proteinase inhibitors.

The importance of maintaining a balance in proteinase inhibitor ratios was dramatically demonstrated more than two decades ago. Ohlsson and Laurell injected two volunteers intravenously with α1PI or α2M less than half saturated with radiolabeled HLE or trypsin (20). Rapid clearance of α2M complexes and α1PI complexes was shown to be mediated by the mononuclear phagocyte system and to be accomplished by transient circulatory changes in some circumstances. Intravenous injection in dogs resulted in shock and death whenever the concentration of active proteinase exceeded that of α2M, even though circulating α1PI was less than half saturated with proteinase (19). The concept that HLE and α1PI might impact HIV infectivity originated following the observations that gp120 exhibits a proteinase-inhibiting domain (1) and that CD4, the T-lymphocyte antigen receptor, elastase, and α1PI are functionally associated during antigen-specific lymphocyte activation (8). Evidence that α1PI and cell surface HLE participate during HIV infectivity (7) (C. L. Bristow, unpublished data) suggested the hypothesis that α1PI or α2M concentrations might be altered during HIV disease.

MATERIALS AND METHODS

Serum specimens. Sera from healthy volunteers were collected after obtaining informed consent. Sera from HIV-infected patients were excess specimens recovered from previous studies for which informed consent had been obtained. HIV RNA in serum was quantitated by PCR using the Amplicor HIV-1 Monitor Test (Roche Diagnostics, Branchburg, N.J.) by the Retrovirology Core Laboratory, University of North Carolina (UNC)—Chapel Hill, using procedures recommended by the manufacturer. Clinical category of disease progression at the time of specimen collection was determined using 1993 Centers for Disease Control and Prevention classification criteria. The clinical parameters examined included HIV RNA, lymphocyte markers, demographic characteristics, known infectious diseases, and antiretroviral therapy. In this study, 44% category A1,A2, 57% category B1,B2, and 58% AIDS patients had or were receiving azidothymidine therapy, and none were receiving HIV-specific protease inhibitors. Characteristics not presented herein were found not to contribute significantly to the analysis. All measurements, chart extraction, and data analysis were performed in a blinded fashion. Data were excluded from analysis if the sera had not been maintained at −80°C prior to analysis, the patient charts could not be located, or the patient charts indicated no evidence of HIV infection.
### TABLE 1. Parameters of HIV disease progression

<table>
<thead>
<tr>
<th>Category (n)</th>
<th>CD4 (cells/μl)</th>
<th>CD8 (cells/μl)</th>
<th>HIV RNA (log10 copies/ml)</th>
<th>Active α1P1 concn (μM)</th>
<th>Inactive α1P1 concn (μM)</th>
<th>Total α1P1 concn (μM)</th>
<th>α2M concn (μM)</th>
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<tr>
<td>Normal (30)</td>
<td>1,415 ± 307</td>
<td>850 ± 203</td>
<td>ND</td>
<td>33.8 ± 12.5‡</td>
<td>2.3 ± 7.2</td>
<td>36.0 ± 13.9</td>
<td>3.36 ± 1.82</td>
</tr>
<tr>
<td>A1,A2 (18)</td>
<td>549 ± 302</td>
<td>927 ± 306</td>
<td>3.16 ± 0.64</td>
<td>12.2 ± 4.5</td>
<td>44.5 ± 40.0</td>
<td>56.7 ± 39.5</td>
<td>2.91 ± 1.35</td>
</tr>
<tr>
<td>B1,B2 (14)</td>
<td>274 ± 50</td>
<td>848 ± 622</td>
<td>3.63 ± 0.65</td>
<td>37.7 ± 28.7</td>
<td>113.5 ± 89.3</td>
<td>151.2 ± 80.5</td>
<td>4.13 ± 2.95</td>
</tr>
<tr>
<td>AIDS (36)</td>
<td>67 ± 63</td>
<td>499 ± 317</td>
<td>4.31 ± 0.78‡</td>
<td>26.5 ± 22.6</td>
<td>31.3 ± 39.2</td>
<td>57.8 ± 39.0</td>
<td>4.51 ± 2.75</td>
</tr>
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</table>

* Data are represented in Fig. 1. Normal values for T-lymphocyte distribution in 100 healthy donors were determined by the Flow Cytometry Laboratory, UNC Hospitals. All other normal values were determined in the sera of 30 healthy donors as described in the text. Values for α1P1 were not normally distributed. A comparison of median values by Kruskal-Wallis analysis of variance on ranks in the normal, asymptomatic, symptomatic pre-AIDS, and AIDS categories of HIV disease revealed significant differences in the concentrations of total (P < 3 x 10^-3), active (P < 2 x 10^-3), and inactive (P < 3 x 10^-8) α1P1 in serum. Median values for each clinical category were compared to normal values by using the Mann-Whitney rank sum test. Individual values and medians are depicted in Fig. 1. Values for HIV RNA were normally distributed. Analysis of variance revealed no significant differences in HIV RNA between categories A1,A2 and B1,B2 and significant differences between categories B1,B2 and AIDS (P < 0.01). Analysis of variance revealed no significant differences in HIV RNA between categories A1,A2 and B1,B2 and significant differences between categories A1,A2 and AIDS (P < 0.0001) and B1,B2 and AIDS (P = 0.0008). Values in boldface are significantly different from normal (P < 0.0001). ND, not detectable.

Quantitation of αM and α1P1 and T-cell markers. Methods for quantitating active αM and α1P1 by determination of elastase inhibitory capacity have been described elsewhere (6). Total α1P1 was determined by enzyme-linked immunosorbent assay (ELISA). Inactive α1P1 was expressed as a percentage of total α1P1 and active α1P1. Measurements of CD4, CD8, and CD3+ levels were performed by the Flow Cytometry Laboratory, UNC Hospitals. Normal values were determined from 100 healthy donors (Janet Mantell and Cindy Evoy, unpublished results). Data were not normally distributed, and all comparisons were made using nonparametric methods. Medians among clinical categories were compared by using the Mann-Whitney rank sum test. Correlation was measured using the Pearson product moment correlation.

**Detection of anti-α1P1 immune complexes.** Detection of anti-α1P1 immune complexes was performed by modification of the α1P1-specific ELISA (6). Sera were heat inactivated for 30 min at 56°C. Anti-α1P1 immune complexes were captured by diluting an aliquot in 0.05 M Tris(hydroxymethyl)aminomethane–0.15 M NaCl (pH 7.8) in wells of a microtiter plate precoated with the immunoglobulin fraction of chicken anti-human α1P1 (lot 188323869; O.E.M. Concepts, Toms River, N.J.), followed by incubation for 120 min at 37°C. In parallel, to detect α1P1 in serum by first dissociating the anti-α1P1 immune complexes, the sera were acidified by diluting an equivalent aliquot in 0.05 M glycine–HCl (pH 3.0). After incubation for 60 min at 37°C, acidified sera were adjusted to pH 7.8, and complexes were allowed to reassociate for an additional 60 min at 37°C. Binding was detected by using horseradish peroxidase (HRP)-complexed rabbit anti-human immunoglobulin G (IgG; lot 091944808; Sigma). Anti-gp120 ELISA. Immune recognition of α1P1 by anti-gp120 was determined by ELISA (6). Wells of a microtiter plate (Nunc) were precoated with various concentrations of α1P1 (A6150; Sigma) or soluble CD4 (Biogen, Cambridge, Mass.). Microtiter plates were incubated for 60 min at 20°C with monoclonal anti-gp120 (1C1, lot 704-111; Repligen, Cambridge, Mass.) in the presence or absence of 1 μg of competing recombinant gp120 (lot 1008-25; Repligen) per ml. Binding was detected with HRP-conjugated protein A (Zymed, South San Francisco, Calif.). Immunorecognition of α1P1 by HRP-conjugated monoclonal anti-gp120 (approximate epitope V3 loop, 10 μg/ml; Research Diagnostics, Flanders, N.J.) was performed for comparison.

**Statistical analysis.** Normality was determined using the Kolmogorov-Smirnov test. Values for CD4, CD8, α1P1, and α2M were not normally distributed, and all comparisons were made using nonparametric methods. Medians among clinical categories were compared by using Kruskal-Wallis one-way analysis of variance on ranks. Comparison of medians between pairs of clinical categories was done by using the Mann-Whitney rank sum test. Values for anti-α1P1 and HIV-1 RNA were normally distributed, and all comparisons were made by using one-way analysis of variance. Comparison of medians between pairs of clinical categories was done by t test. Correlation was measured using Pearson product moment correlation, and linear regression was measured by computer-fit least-squares analysis.

**RESULTS**

α1P1 levels in clinical category of HIV disease. The α1P1 and α2M levels were quantitated in the sera from 68 HIV-seropositive patients and 30 seronegative volunteers (Table 1). None were being treated with HIV-specific protease inhibitors at the time of blood collection. In the normal subjects studied here, the mean value for total α1P1 was 36.0 ± 13.9 μM and ranged from 18 to 53 μM between the 5th and 95th percentiles (Fig. 1). The mean α2M concentration in the present study was 3.36 ± 1.82 μM and ranged from 1.26 to 5.57 μM between the 5th and 95th percentiles. Values for α1P1 and α2M determined here are consistent with previous studies (5, 12).

The total concentration of α1P1 in an individual serum sample represents a unique mixture of active and inactive α1P1. In health, the active concentration has been found to represent 90 to 100% of total α1P1 concentration (6). In the normal subjects studied here, the mean value for active α1P1 was 33.8 ± 12.5 μM and ranged from 18 to 48 μM between the 5th and 95th percentiles. The mean value for inactive α1P1 was 2.3 ± 7.2 μM and ranged from 0 to 11 μM between the 5th and 95th percentiles. Consistent with previous studies, population values were found not to be normally distributed, necessitating statistical comparisons using medians (5). Comparison of normally healthy subjects, asymptomatic (clinical categories A1 and A2), symptomatic pre-AIDS (clinical categories B1 and B2), and AIDS (clinical categories A3, B3, and C1 to C3) categories of HIV disease revealed significant differences in the concentrations of total (P < 3 x 10^-3), active (P < 2 x 10^-3), and inactive α1P1 (P < 3 x 10^-8) in serum but not of α2M (P < 0.3) (Fig. 1).

During the acute phase of inflammation, total α1P1 can increase as much as fourfold. As would be expected, total α1P1 was significantly elevated in 100% of symptomatic pre-AIDS patients but was not different from the normal level in the asymptomatic HIV population. Values were considered elevated if they exceeded the 95th percentile and deficient if they fell below the 5th percentile of the normal range. Total α1P1 was elevated in 33% and deficient in 11% asymptomatic patients. Surprisingly, total α1P1 in AIDS was not significantly different from normal. In this group, 42% were elevated and 22% were deficient. Review of the spectrum of infections documented at the time of blood collection revealed no significant trends (data not shown).

Unexpectedly, the concentration of active α1P1 was found to be deficient in 100% of asymptomatic, 44% of symptomatic pre-AIDS, and 56% of AIDS patients (Fig. 1). At concentra-
tions of active H9251 PI of H11022 M, the odds were 11:1 that CD4 H9262 cell counts were 300 cells/l (P = 0.01) in the HIV-seropositive population. As would be expected, active H9251 PI was elevated in 29% of symptomatic pre-AIDS patients and in 11% of AIDS patients.

In the asymptomatic category of disease, increased HIV RNA was found to be correlated with increased total H9251 PI (P = 0.04) but not correlated with CD4 levels. Further, HIV RNA was neither correlated with CD4 levels nor total H9251 PI in symptomatic and AIDS patients. Increased HIV RNA was found to be correlated with decreased CD4 levels when all clinical categories were included in the analysis (P = 0.00005).

These results support previous findings that decreased H9251 PI is associated with decreased HIV production. Comparison of the sensitivities and specificities of H9251 PI, CD4, and HIV RNA measurements in determining the clinical category of HIV disease in the patient population represented here suggests that measuring H9251 PI could potentially provide a significant improvement in defining clinical course (Table 2).

Inactive H9251 PI was found to be elevated in 78% of asymptomatic, in 86% of symptomatic pre-AIDS, and in 64% of AIDS patients. The etiologic mechanisms of H9251 PI inactivation might involve proteolytic inactivation, oxygenation, diminished synthesis, and autoimmune reactivity. A state of dysregulated proteolysis has been shown to exist in HIV disease (23), and this results in generalized diminution in circulating competent protease inhibitors.

Like H9251 PI, H9251 M can be inactivated by protease interaction, as well as by oxygenation. In support of uncontrolled proteolysis, among symptomatic pre-AIDS patients 40% were elevated and 30% were deficient in active H9251 M concentration. However, 80% of asymptomatic patients and 76% of AIDS patients were within the normal limits for H9251 M, and this reflects a disposition of controlled protease activity in these

![Fig. 1.](http://cvl.asm.org/) α1PI and α2M concentrations in serum by clinical category of HIV disease. (A) Values were not normally distributed. Category medians are represented by bars and are statistically compared in Table 1. (B) HIV RNA was found to be correlated with total α1PI in asymptomatic patients (P < 0.04) but not in other clinical categories. HIV RNA was not related to CD4 levels in any clinical category. (C) There was no relationship between α2M and α1PI concentrations in asymptomatic and symptomatic pre-AIDS patients; however, in AIDS patients, α2M was correlated with both active α1PI (r² = 0.73, P < 6 × 10⁻⁶) and inactive α1PI (r² = 0.45, P < 0.04). (D) Schematic representation of CD4 (- - - -), HIV RNA (-), active α1PI (-----), and total α1PI (––––) concentrations in this patient population.

**TABLE 2. Sensitivities and specificities of α1PI, CD4, and HIV RNA as prognostic indicators for transition between clinical categories in HIV disease**

<table>
<thead>
<tr>
<th>Test</th>
<th>Disease progression</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total α1PI</td>
<td>A1,A2 to B1,B2</td>
<td>77.8</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>B1,B2 to AIDS</td>
<td>100</td>
<td>80.6</td>
</tr>
<tr>
<td>CD4</td>
<td>A1,A2 to B1,B2</td>
<td>83.3</td>
<td>92.9</td>
</tr>
<tr>
<td></td>
<td>B1,B2 to AIDS</td>
<td>100</td>
<td>97.2</td>
</tr>
<tr>
<td>HIV RNA</td>
<td>A1,A2 to B1,B2</td>
<td>66.7</td>
<td>78.6</td>
</tr>
<tr>
<td></td>
<td>B1,B2 to AIDS</td>
<td>85.7</td>
<td>69.4</td>
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</table>

*Sensitivities and specificities were determined from the data represented in Fig. 1 using CART (classification and regression tree) analysis by the Biostatistics Core of the UNC Center for AIDS Research.*
clinical categories. The α₂M concentrations in HIV disease, including all clinical categories, were not significantly different from normal, and this suggests that deficient levels of α₁PI could not be completely explained by dysregulated proteolysis nor oxygenation. Significantly, in AIDS, but not in other clinical categories of disease, α₂M levels were correlated with active α₁PI (P < 0.003) and with inactive α₁PI (P < 0.004), suggesting that mechanisms other than dysregulated proteolysis were involved in producing increased levels of inactive α₁PI in the asymptomatic category. Further, the recovery in the circulating active α₁PI concentration during the symptomatic stages of disease suggested that the synthesis of α₁PI may not be impaired and that alternative processes could be responsible for acquired deficiency. A previous study has demonstrated no difference in α₁PI synthesis during AIDS in contrast to an increased synthesis of the acute-phase C-reactive protein (11).

**Anti-α₁PI in HIV disease.** Since autoantibodies recognizing multiple cytokines and cellular proteins are known to occur in HIV disease (14), the potential for an autoimmune process in producing decreased active α₁PI was examined. Antibodies recognizing immobilized α₁PI were not discernible in serum from this patient population by traditional ELISA (data not shown). However, it was found that α₁PI-anti-α₁PI immune complexes were detectable by capturing them with immobilized anti-α₁PI and detecting them with anti-human IgG. Control sera from 17 normal subjects and 1 subject with inherited α₁PI deficiency were found to have background values of 11.2 ± 8.4 milliabsorbance at 405 nm (m405/min) and to range between 0.9 and 26.0. In contrast, 56% of asymptomatic, 57% of symptomatic pre-AIDS, and 31% of AIDS patients exceeded the mean control value by 2 standard deviations (Table 3). The high concentration of α₁PI in serum suggested that the appearance of anti-α₁PI would rapidly produce immune complexes, precluding detection by this method. It was further considered that immune complex formation might obscure α₁PI epitopes, preventing capture using anti-α₁PI and resulting in underestimation. We have previously shown in insulin-dependent diabetes mellitus that insulin-anti-insulin immune complexes are obscured in this manner (28). We determined that acidification of serum allows dissociation of immune complexes and that neutralization of acidified serum in the presence of competing radiolabeled ligand allows the detection of insulin–anti-insulin immune complexes. Using a modification of this approach, acidification and neutralization of sera in the presence of immobilized competing anti-α₁PI allowed detection of α₁PI-anti-α₁PI immune complexes in an additional 33% of asymptomatic patients, an additional 7% of symptomatic pre-AIDS, and an additional 14% of AIDS patients. Background values (m405 per minute) in normal control sera were not influenced by acidification and neutralization. Overall, 89% of asymptomatic, 64% of symptomatic pre-AIDS, and 44% of AIDS patients had detectable anti-α₁PI IgG, and this decline in anti-α₁PI IgG is consistent with the decline in specific antibody response during AIDS. An increased anti-α₁PI level was correlated with decreased active α₁PI in the asymptomatic category of HIV disease (P < 0.05). Increased anti-α₁PI was correlated with increased inactive α₁PI in symptomatic (P < 0.03) and AIDS patients (P < 0.03), and this suggests that α₁PI autoantibodies are responsible in part for acquired α₁PI deficiency. In patients with detectable anti-α₁PI IgG immune complexes, the odds were 36.1 that active α₁PI would be deficient (P < 0.01). Further, in AIDS patients, the mean reactivity (m405 per minute) was significantly lower than in the other clinical categories (P < 0.002, Fig. 2), and this result is consistent with the decline in detectable anti-α₁PI. The correlation between α₂M and active and inactive α₁PI in AIDS suggests that infection-related dysregulated proteolysis also contributes to the deficiency in α₁PI detected.

**TABLE 3. Circulating α₁PI-specific IgG immune complexes in HIV disease**

<table>
<thead>
<tr>
<th>Category (n)</th>
<th>Anti-α₁PI IgG m405/min ± SD (%)</th>
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<tbody>
<tr>
<td>A1, A2 (18)</td>
<td>Negative: 8.7 ± 5.7 (50)</td>
</tr>
<tr>
<td></td>
<td>Positive: 36.8 ± 14.8 (44)</td>
</tr>
<tr>
<td></td>
<td>Negative and positive: 21.2 ± 17.7</td>
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</table>

**Note:**

- Values were scored as positive if they exceeded 2 standard deviations above the mean value detected in 17 normal control sera at pH 7.8 (11.2 ± 8.4 m405/min) or at pH 3.0 (9.5 ± 6.1 mOD/min).
- Means were normally distributed. Comparison of mean values by analysis of variance revealed significantly lower values in AIDS patients than in asymptomatic (P < 0.0005) or symptomatic pre-AIDS (P < 0.03) patients. Actual m405/min values were correlated with CD4⁺ cell counts (P < 0.008) and CD8⁺ cell counts (P < 0.002) in the HIV-seropositive patients and with α₁PI deficiency (P < 0.05) in the asymptomatic category. The frequency of patients positive for anti-α₁PI IgG in each category was correlated with CD4⁺ cell counts (r² = 0.999, P < 0.01) but not with CD8⁺ cell counts (P < 0.2). Data are represented in Fig. 2.

**FIG. 2. Autoantibodies reactive with α₁PI in HIV disease.** (A) Autoantibodies reactive with α₁PI were detected by ELISA. Bars represent m405 per minute and are statistically compared in Table 3. The mean reactivity was significantly lower in AIDS patients than in asymptomatic (P < 0.0005) or symptomatic pre-AIDS (P < 0.02) patients. (B) A monoclonal antibody with specificity for gp120 epitopes near the fusion domain also recognized α₁PI (●) but not CD4 (■). Binding of anti-gp120 to α₁PI was inhibited in the presence of 8 μM competing gp120 (●). A monoclonal antibody with specificity for gp120 epitopes near the V3 loop failed to recognize α₁PI (○). (C) Alignment of amino acid sequences for human α₁PI and the epitope recognized by anti-HIV gp120 near the fusion domain revealed significant homology. Boxed residues are identical or conservatively substituted. The immunizing peptide for monoclonal anti-gp120 antibody is underlined. The hydrophobic α₁PI pentapeptide is depicted in boldface. The proteinase reactive site of α₁PI is Met358. HIV gp120 and gp41 are produced by cleavage near Lys517 and Arg518 to reveal the fusion domain.
Significantly, as is true for viral RNA, the actual mI4s/h per minute values for calculating anti-αPI IgG immune complexes, including all clinical categories, were correlated with CD4+ cell counts (P < 0.003) and CD8+ cell counts (P < 0.03). The frequency of patients in each clinical category having circulating anti-αPI IgG immune complexes was inversely related to CD4+ cell counts (r² = 0.999, P < 0.01) but not with CD8+ cell counts (P < 0.2; data not shown). These data suggest that in symptomatic pre-AIDS and AIDS, lymphocyte population abnormalities may be responsible in part for the decline in αPI autoantibody antibodies.

Evidence that the V3 loop of gp120 contains a domain identical to the active site of InI and inhibits proteolytic activity in certain circumstances (1) suggested that gp120 and αPI might share antigenic epitopes. To determine the potential for antigenic mimicry, immunoreactivity for αPI by antibodies specific for gp120 was examined by ELISA. Significant binding to αPI was detected by antibodies recognizing epitopes near the fusion domain but not epitopes near the V3 loop. Binding of anti-gp120 to αPI was competitively inhibited by gp120, suggesting that molecular mimicry may explain this epitope specificity. Comparative alignment of the carboxyl terminus amino acid sequence of αPI and the HIV peptide sequence recognized by the immunoreactive monoclonal antibody revealed considerable homology. Significantly, these sequences are flanked by the hydrophobic chemotactic pentapeptide domain of αPI (FXFXX, where X = V, L, I, or M) and the hydrophobic the HIV fusion domain (FLGFL). This result suggests that in the HIV-seropositive patient population, antibodies with specificity for gp120 might also recognize αPI potentially diminishing the active αPI concentration.

**DISCUSSION**

We unexpectedly found in this study that active αPI was deficient in all asymptomatic patients. On the other hand, total αPI was elevated in all symptomatic pre-AIDS HIV-positive patients. The strong association between αPI and disease progression suggested αPI might be central to the pathophysiology of HIV disease. Combined with CD4+ levels, determining the viral load (plasma viral RNA) is integral to the recommended guidelines for the clinical management of HIV disease. Discordant values for plasma viral RNA and CD4+ cell counts occur in approximately 14% of patients receiving antiretroviral therapy, and this complicates therapeutic decisions. The prognostic value of αPI concentration for determining clinical category of HIV disease was equivalent to that of CD4, and this finding supports recent evidence that this self antigen acts as an entry cofactor.

More than 75 codominant alleles of human αPI have been identified that are correlated in mucosal secretions and serum (16). In 1,084 plasma samples representing six species of monkeys (Macaca irus, M. mulatta, M. cyclopis, M. nemestrina, M. speciosa, and M. fuscata), five codominant alleles have been identified (3). These genetic differences might serve as a key to understanding the different pathologic outcomes of HIV infection in humans and chimpanzees. Neither antibodies to αPI nor deficiency in αPI were detected in macaques infected with simian/human immunodeficiency virus (SHIV) (data not shown), suggesting that different pathophysiologic outcomes result during HIV disease in humans and SHIV disease in macaques.

The primary source of circulating αPI is thought to be the liver. Synthesis and secretion of αPI by lymphocytes, mononuclear phagocytes, cornea, and intestinal Paneth cells in response to interleukin-6, transforming growth factor β, granulocyte-macrophage colony-stimulating factor, HLE, and lipopolysaccharide from gram-negative bacteria is under the regulation of NF-κB (22). Convincing evidence from αPI phenotype-mismatched patients undergoing bone marrow transplantation suggests that the mononuclear phagocyte system does not contribute to the systemic pool (13). However, it can be speculated that αPI secreted by the mononuclear phagocyte system might explain the low levels of αPI in patients homozygous for the PI2z phenotype who lack the capacity for hepatocyte secretion of αPI. It can be further speculated that αPI secreted by cells of the mononuclear phagocyte system might primarily remain tissue associated, creating a localized gradient.

The distribution of human αPI phenotypes has been reported to differ between ethnic groups and between groups dichotomized by sexual preference (9). Testosterone can induce a 20-fold increase in αPI mRNA, and during pregnancy αPI increases by 100% and αM increases by 20% (10). The detection of anti-αPI IgG in HIV-seropositive patients suggests that autoimmune recognition of αPI may be one determinant in producing αPI deficiency. This result further suggests that in the presence of competing anti-αPI immune complexes, the quantitation of total and inactive αPI by ELISA in these patients may have been underestimated here. The binding of αPI by monoclonal anti-gp120 suggests humoral immunity to gp120 is one potential mechanism for initiating an autoimmune response to αPI. Comparative alignment of the antibody-recognized gp120 peptide sequence and the carboxyl-terminal amino acids of αPI supports antigenic mimicry. Further, because of the hydrophobic nature of the αPI epitope recognized by anti-gp120, these results suggest that neo-epitopes of αPI may be exposed during HIV disease. It has been suggested that viral proteins interactive with host proteins can induce conformational changes and the exposure of neo-epitopes, thereby initiating autoimmunity (26), and this suggests a potential mechanism for the initiation of autoimmunity to αPI. Antibodies recognizing the V3 loop have been shown to inhibit the activity of InI (18), and this suggests that the functional activity of InI may also be diminished in the HIV-seropositive population. Deficiency or overexpression of InI has not been described in any disease process (4); however, the active derivative of InI has been reported to localize to affected tissue in Alzheimer-type dementia (29) and to increase during bacterial infection (24). That morbidity and mortality are documented to result from an imbalance in αPI and αM ratios (19, 20) suggests that autoimmunity to αPI may be cardinal to HIV-associated pathophysiology. The HIV-induced autoimmune recognition of αPI raises an important concern regarding vaccine development. It is not known at this time whether the initiation of immunity to HIV by vaccination also may initiate autoimmunity to αPI. Of considerable interest is that immune recognition of multiple HIV proteins can be detected in HIV-exposed seronegative individuals; however, neither IgA nor IgG with specificity for gp120 could be de-
ected in these individuals (15). The evidence from this continuing seronegative population for the potential to coexist with HIV suggests, therefore, that protection against AIDS in HIV-infected individuals might arise by preventing autoimmunity to α1PI.

Results presented elsewhere suggest that α1PI deficiency, a condition which exists in the asymptomatic category of HIV disease, may promote increased cellular sensitivity to HIV infectivity (8a). On the other hand, the increasing concentrations of α1PI which occur during bacterial infection may promote increased viral load, conditions known to occur during the symptomatic category of HIV disease. The correlation between HIV RNA and total α1PI in the symptomatic category supports the hypothesis that changing concentrations of circulating α1PI in response to HIV proteins and in response to attendant infections during HIV disease directly impact HIV pathophysiology. Evidence that plasma membrane-associated HLE has been previously shown to interact with gp120 (17) and recent evidence that neither cell lines nor peripheral blood mononuclear cells infected in vitro produce detectable RT activity in the absence of α1PI (8a) support the participation of these proteins during HIV disease.

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REFERENCES