The Clinical Significance of Human Immunodeficiency Virus Type 1-Associated Paraproteins

By Valerie L. Ng, Katherine H. Chen, Kou M. Hwang, Hassan Khayam-Bashi, and Michael S. McGrath

We observed and characterized paraproteins present in the serum of seven human immunodeficiency virus type 1 (HIV-1)-infected individuals. Immunoglobulin (Ig) subclass typing performed on these paraproteins identified five as IgG, κ, one as an IgG3 λ, and one as an IgA λ. The IgG, κ paraproteins, purified by high-pressure liquid chromatography, contained the majority of anti–HIV-1 antibody reactivity present in the five serum specimens (ranging from 1:5,000 to 1:500,000) as demonstrated by immunoblot. All five IgG, paraproteins had at least two light chain species as demonstrated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and the antibodies were reactive with multiple HIV-1 viral antigens. In contrast, the electrophoretically purified IgG3 λ and IgA λ paraproteins did not react with HIV-1 antigens and only one light chain species was detected by SDS-PAGE. The subsequent clinical evaluation of these patients following the initial observation of paraproteinemia failed to correlate the presence of paraproteins with the development of lymphoma over a 2 to 3 year period. These data support the hypothesis that IgG, paraproteins present in the sera of HIV-1 infected individuals reflect a normal albeit exuberant polyclonal immune response to HIV-1 viral antigens. In contrast, the clinical significance of an IgG3 λ or an IgA λ paraprotein is unclear at present.

Hypogammaglobulinemia, often associated with paraproteinemia, is a common observation following infection with the human immunodeficiency virus type 1 (HIV-1). These paraproteins can be detected at all stages of disease associated with HIV-1 infection, ranging from the infected but asymptomatic stage to the development of symptoms associated with acquired immunodeficiency syndrome (AIDS) related complex (ARC) or the development of opportunistic infections diagnostic of AIDS. The mechanism(s) responsible for the induction of HIV-1-associated paraproteins is yet unclear, but may be attributable to the polyclonal B cell activation and lack of B-cell immunoregulation following HIV-1 infection.11-14

Previously we had extensively characterized a paraprotein from an HIV-1 infected individual with ARC, and demonstrated that the paraprotein was polyclonal in origin, reacted with multiple HIV-1 antigens, and contained the majority of anti–HIV-1 antibody activity present in the patient’s serum.15 We had hypothesized that the presence of this paraprotein represented a normal, albeit vigorous, immune response to HIV-1 infection. To further test this hypothesis and to determine if our original finding was applicable to all HIV-1-associated paraproteins, we chose to fully characterize the clinical course of these patients. In an attempt to address all of the points raised above, we present here the full characterization of seven HIV-1-associated paraproteins as well as the partial clinical course of these patients.

METHODS AND MATERIALS

Reagents. Sheep antisera with specific reactivity directed against the heavy (H) chains of each of the different human IgG subclasses was purchased from Jansen Life Sciences Products (Piscataway, NJ). Affinity purified biotinylated rabbit-anti-sheep (heavy and light chain [H + L chain]) antisera and biotinylated goat-anti-human-IgG (γ chain-specific) antisera was purchased from Zymed Laboratories, Inc (South San Francisco, CA); and biotinylated goat-anti-human IgG (H + L chain) was purchased from BioRad Laboratories (Richmond, CA) and the Dade normal human serum controls for serum protein electrophoresis were purchased from American Scientific Products (Baxter Travenol Co, Miami, FL).

Source of patient sera. Sera containing paraproteins were identified when they were submitted for laboratory testing as part of the laboratory evaluation of a patient with an elevated globulin level. Only sera from patients with risk factors for HIV-1 infection or known to be HIV-1 antibody seropositive were selected for this study.
study. The serum from patient H was received from an outside hospital for consultation.

**Serum protein electrophoreses and immunofixation electrophoreses.** Serum protein electrophoreses (SPEPs) and immunofixation electrophoreses (IFEs) were performed as previously described. The anode is to the left, and the cathode is to the right. Lane A shows the SPEP pattern of a normal control serum (Dade). Lanes B through H show the various paraprotein patterns seen in HIV-1 infected individuals. The application site is the clear slot near the right end of each lane: the fractions in lane A, reading from left to right, correspond to albumin, alpha 1, beta 1, the fractions in lane A, reading from left to right, correspond to albumin, alpha 2, beta 1, and gamma.

**RESULTS**

The seven paraproteins characterized in this study are shown in Fig 1. One major paraprotein was detected in the samples shown in lanes C, E, F, G, H; three oligoclonal proteins migrating cathodal to the application site were detected in the sample shown in lane D, and three oligoclonal proteins were detected in the sample shown in lane B—one of which migrated cathodal and two of which migrated anodal to the application site.

The immunoglobulin subclass identity of these paraproteins is shown in Table 1. Only the prominent paraprotein migrating cathodal to the application site in lane B could be subclass typed as an IgG1; the remaining two paraproteins in

Table 1. Laboratory Parameters of Patients With HIV-1–Associated Paraproteins, and the Number, IgG Subclass Typing, and Immunologic Specificities of HIV-1–Associated Paraproteins

<table>
<thead>
<tr>
<th>Patient*</th>
<th>Globulin Level (g/L)</th>
<th>Paraprotein Number</th>
<th>Type</th>
<th>HIV-1 Titer†</th>
<th>Total Paraprotein</th>
<th>HIV-1 Ag Reactivity (Kd)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>65</td>
<td>3</td>
<td>IgG1α</td>
<td>5 x 10⁴</td>
<td>1 x 10⁴</td>
<td>17, 24, 53, 55, 66</td>
</tr>
<tr>
<td>C</td>
<td>88</td>
<td>1</td>
<td>IgG1α</td>
<td>5 x 10⁵</td>
<td>1 x 10⁴</td>
<td>24, 53, 55, 66</td>
</tr>
<tr>
<td>D</td>
<td>60</td>
<td>3</td>
<td>IgG1α</td>
<td>1 x 10⁴</td>
<td>1 x 10⁴</td>
<td>24, 55, 66</td>
</tr>
<tr>
<td>E</td>
<td>43</td>
<td>1</td>
<td>IgG1α</td>
<td>1 x 10⁴</td>
<td>1 x 10⁴</td>
<td>24, 55, 66</td>
</tr>
<tr>
<td>F</td>
<td>41</td>
<td>1</td>
<td>IgG1α</td>
<td>5 x 10³</td>
<td>1 x 10³</td>
<td>17, 24, 41, 55, 66</td>
</tr>
<tr>
<td>G</td>
<td>51</td>
<td>1</td>
<td>IgG3 λ</td>
<td>5 x 10³</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>H</td>
<td>71§</td>
<td>1</td>
<td>IgA λ</td>
<td>5 x 10³</td>
<td>0</td>
<td>None</td>
</tr>
</tbody>
</table>

Abbreviation: Ag, antigen.

*Letters correspond to lanes seen in Fig 1.

†HIV-1 titer determined by immunoblot. Total refers to total anti–HIV-1 antibody reactivity present in serum/plasma; paraprotein refers to total anti–HIV-1 antibody reactivity present in the purified paraproteins.

‡Reactivity of the purified paraproteins with various HIV-1 antigens. Products of the gag gene are 55, 24, and 17 Kd; products of the pol gene are 66, 53, and 31 Kd; products of the env gene are 160, 120, and 41 Kd.

§47.3 g/L of this serum globulin level was contributed by the IgA λ paraprotein.
this sample that migrated anodal to the application site were not present in quantities sufficient for IgG subclass typing.

Of interest was the finding that five of the seven paraproteins were of the IgG, k subclass. All of these sera had high titer anti-HIV-1 antibody activity, the majority of which could be localized to the purified paraprotein fraction. In contrast, the patients whose SPEPs are shown in lanes G and H had an IgG, O and an immunoglobulin A (IgA) L paraprotein, respectively.

We examined the purified paraproteins by SDS-PAGE as a crude method of determining clonality of the paraproteins. If multiple L chain species were detected, it supported the hypothesis that the paraproteins were polyclonal in origin; if a single L chain was observed, it suggested that the paraprotein might be monoclonal in origin. All of the IgG, paraproteins had at least two L chain species present in the purified paraproteins demonstrated by SDS-PAGE. The electrophoretically purified IgA K paraprotein had only one L chain demonstrated by SDS-PAGE (data not shown).

The electrophoretically purified IgG, paraprotein also had two L chain species by SDS-PAGE analysis, one of which was the predominant species. However, electrophoretic purification could not separate the paraprotein from background polyclonal immunoglobulins with similar electrophoretic charge; therefore, the finding of more than one L chain species by SDS-PAGE was expected. The fact that one L chain was present in much greater quantities suggested that this L chain species was associated with the paraprotein. A later serum sample obtained from this same patient after the loss of his paraprotein, analyzed in the same manner, demonstrated the absence of this previously predominant L chain species, further substantiating its paraprotein association.

The HIV-1 antigen reactivity of total serum/plasma versus that of the purified paraproteins is shown in Table 1. All of the IgG, K paraproteins had reactivity with gag (p17, p24, and p55) and pol (p66 and p53) determinants. Although the total serum/plasma reactivity for each of these patients also contained reactivity against env gene products (gp41, gp120 and gp160), such reactivity could not be demonstrated in the purified paraproteins. The serum containing the IgG, L paraprotein had reactivity with pol and env gene products; however, a later serum sample obtained after the disappearance of the paraprotein still retained anti-HIV antibody reactivity at the same titer and directed against the same HIV proteins. We thus concluded that the IgG, L paraprotein did not contribute to the total anti-HIV antibody reactivity in this patient’s serum and thus was not directed against HIV-1 antigens. The IgA L paraprotein completely lacked reactivity with HIV-1 antigens.

The partial clinical course of these patients, subsequent to and at the time of the initial observation of paraproteinemia, is detailed in Table 2. Patients B and E were lost to follow-up. Patient C (whose complete clinical history is presented elsewhere) had no progression of his clinical disease. Patient D, although suffering two successive bouts of bacterial pneumonia, had not progressed to either ARC or AIDS 3 years after the initial observation of paraproteinemia. Patient F, who had a primary AIDS diagnosis of lymphoma when his paraproteinemia was initially observed, had a complicated course and ultimately succumbed to Pneumocystis carinii (P. carinii) pneumonia. Patient G, whose complete clinical history was reported elsewhere,17 lost his paraprotein coincident with the development of primary biliary lymphoma. No further clinical information was available for patient H. In all patients who had subsequent clinical and laboratory evaluations, serum globulin levels were still elevated at the time of their most recent visit.

DISCUSSION

We have presented the laboratory and clinical findings of seven patients with HIV-1–associated paraproteins. Five of the HIV-1–associated paraproteins were IgG, K. These IgG, paraproteins, of which the exhaustive characterization of one
has been reported previously (patient C)\textsuperscript{3} were all polyclonal in origin, as demonstrated by multiple L chain species separated on SDS-PAGE and by the multiple HIV-1 antigen specificities recognized by the purified paraproteins. In contrast, the properties of the IgG\textsubscript{3} and IgA paraproteins were different from those of the IgG\textsubscript{1} paraproteins, in that only a single L chain species was detected by SDS-PAGE, and that the purified paraproteins did not react with HIV-1 antigens. Thus, our original hypothesis that HIV-1–associated paraproteins reflect a vigorous and normal immune response to HIV-1 infection is only applicable to IgG\textsubscript{1} paraproteins.

The IgG\textsubscript{3} and IgA\textsubscript{1} paraproteins must be considered in a different category than the IgG\textsubscript{1} paraproteins. Neither of these paraproteins recognized HIV-1 antigens and their antigenic specificity remains unknown. These paraproteins appear to be monoclonal based on indirect tests (one L chain demonstrated by SDS-PAGE; one single L chain species demonstrated by IFE). Unfortunately, we were unable to obtain tissue to perform immunoglobulin gene rearrangement studies to definitively address the issue of clonality. The lack of subsequent clinical information on the patient with the IgA\textsubscript{1} paraprotein, and the fact that we have only identified one patient with an IgG\textsubscript{3} paraprotein does not allow us to assess the clinical significance of these paraproteins.

Of the five patients with HIV-1–associated IgG\textsubscript{1} paraproteins, one was lost to follow-up, two have remained clinically stable, one has had two successive bouts of bacterial pneumonia with complete recovery, and one has died from \textit{P carinii} pneumonia. All of these patients continued to have persistently elevated globulin levels during their subsequent clinical evaluations. Repeat SPEPs have only been requested for patients C and D; the amount and identity of the paraproteins in these two individuals has remained constant over the 3 to 4 years of follow-up. Repeat SPEPs were not ordered on the most recent visits of the other patients described here; we can only assume that their paraproteins were still present by virtue of their persistently elevated globulin levels.

Recent studies have found that there is a restricted IgG subclass response to HIV-1 infection, such that antibodies directed against \textit{gag} proteins were found in all the IgG subclasses (but predominantly in the IgG\textsubscript{1} and IgG\textsubscript{3} subclasses),\textsuperscript{18,19} antibodies directed against the pol gene products were found in the IgG\textsubscript{1} and IgG\textsubscript{3} subclasses, and that antibodies directed against \textit{env} gene products were restricted to the IgG\textsubscript{1} subclass.\textsuperscript{19} Antibody response to HIV-1 also varies with the clinical stage of disease, such that anti–HIV-1 antibodies of all IgG subclasses have been seen in asymptomatic HIV-1 infected individuals; however, only IgG\textsubscript{1} and IgG\textsubscript{3} anti–HIV-1 antibodies are detected in AIDS patients.\textsuperscript{19} Our finding of five polyclonal IgG\textsubscript{3} \& paraproteins with antibody reactivity against multiple \textit{gag} \& \textit{pol} antigens is consistent with these previous reports, and adds further support to our hypothesis that IgG\textsubscript{3} paraproteins reflect a vigorous and normal immune response to HIV-1 infection.

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REFERENCES


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