High Titer Anti-HIV Antibody Reactivity Associated With a Paraprotein Spike in a Homosexual Male With AIDS Related Complex

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We observed a human immunodeficiency virus (HIV)-infected homosexual male with AIDS related complex (ARC) who had a serum globulin level of 80 g/L. Serum protein electrophoresis revealed a gamma globulin fraction of 40 g/L, of which 50% (20 g/L) was contained within a paraprotein spike, comprised predominantly of IgG kappa. This patient also had high titer anti-HIV antibodies in his serum, which were Western blot reactive at a final dilution of 1:500,000, and recognized gpl20\(^{\text{gp}}\), p66\(^{\text{gp}}\), p55\(^{p}\), p53\(^{p}\), p41\(^{p}\), and p24\(^{p}\). Because paraproteins in the past have been shown to be directed against specific antigens, we purified this patient’s paraprotein using a modified high performance liquid chromatography (HPLC)-hydroxylapatite procedure and tested the purified paraprotein for anti-HIV antibody activity. The purified paraprotein retained anti-HIV antibody activity to a final dilution of 1:100,000, and recognized p66\(^{\text{gp}}\), p55\(^{p}\), p53\(^{p}\), p41\(^{p}\), and p24\(^{p}\). The recognition of both “gag” and “pol” gene products suggested that the purified paraprotein might not be monoclonal in origin. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) demonstrated that the purified paraprotein contained at least two immunoglobulin light chain species (Mol wt 30 to 33 Kd). Affinity chromatography of the purified paraprotein using a p24-Sepharose 4B matrix separated the “gag” and “pol” antibody activities. Immunoglobulin gene rearrangement analysis of a bone marrow aspirate (which contained 15% plasma cells) failed to reveal a clonal population of immunoglobulin producing cells. We conclude that this patient’s paraprotein accounted for most of the anti-HIV activity present in whole serum, and that this paraprotein was not monoclonal in origin.

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A N EARLY manifestation of infection with the human immunodeficiency virus (HIV) is hypergammaglobulinemia, often accompanied by high titer anti-HIV antibody activity.\(^1\) Recently, a subset of AIDS patient sera have been found to contain oligoclonal and/or monoclonal paraproteins.\(^2\) These paraproteins were found in sera from AIDS patients with Kaposi’s sarcoma and with opportunistic infections, but were not associated with a clonal B cell process such as multiple myeloma. The mechanism responsible for induction of these paraproteins is unclear but may be related to polyclonal B cell activation and aberrant B cell immunoregulation following HIV infection.\(^3\)^\(^2\)^\(^2\)^\(^4\)

Infectious disease associated benign and transient paraproteins have been observed in sera from individuals with congenital toxoplasmosis, malaria, congenital syphilis, Pseudomonas meningitis, cytomegalovirus infection, and subacute sclerosing panencephalitis.\(^9\)^\(^1\)\(^1\) The transient nature of some of the paraproteins associated with these infections strongly implicates the presence of an extrinsic antigenic stimulus driving their production. However, to date, no specific antigen or set of antigens has been directly implicated in causing these transient paraproteinemias. We wished to determine whether an AIDS-associated paraprotein might be directed against HIV antigenic determinants.

Paraproteinemias that are found to be non-transient in nature generally require an evaluation for the presence of a B cell malignancy (i.e., multiple myeloma) Because high-grade non-Hodgkin’s B cell lymphoma occurs at a relatively high frequency in HIV-infected individuals, we also wished to determine whether HIV induced the production of paraproteins and/or such paraproteins would be the product of a clonal B cell process. In this communication we describe an HIV infected homosexual male with AIDS related complex (ARC) who had a serum globulin level of 80 g/L. Serum protein electrophoresis (SPEP) revealed a gamma globulin fraction of 40 g/L, of which 20 g/L was contained within an IgG kappa paraprotein spike. The high performance liquid chromatography (HPLC) purified paraprotein contained most of the total anti-HIV antibody activity (as determined by Western blot reactivity) present in his serum. We also demonstrated that his purified paraprotein was not monoclonal in origin.

MATERIALS AND METHODS

Case history. RM is a 20-year-old homosexual white man who presented November 1985, with a 1-year history of fatigue, lymphadenopathy, and intermittent night sweats. Physical examination revealed oral hairy leukoplakia and diffuse lymphadenopathy. A chest x-ray was unremarkable. Laboratory abnormalities included a hemoglobin of 11.9 g/dL, a platelet count of 58,000/\(\mu\)L, an erythrocyte sedimentation rate of 103 mm/h, a total serum protein of 112 g/L, and a globulin level of 75 g/L. He was also HBSAg positive and HIV seropositive. His serum anti-HIV antibodies reacted with gpl20\(^{\text{gp}}\), p66\(^{\text{gp}}\), p55\(^{p}\), p53\(^{p}\), p41\(^{p}\), and p24\(^{p}\) (as determined by Western blot), and was reactive to a final dilution of 1:500,000 (data not shown). SPEP revealed a polyclonal hypergammaglobulinemia, on which was superimposed a protein spike migrating cathodal to the application site. Densitometric scanning (not shown) revealed that his paraprotein accounted for approximately 50% of his gamma globulin fraction. Immunofixation electrophoresis (IFE) demonstrated that the paraprotein was comprised predominantly of IgG.
kappa, with trace amounts of IgG lambda. Quantitative serum immunoglobulin measurements revealed an IgG level of 71 g/L (4.23 to 16.85 g/L), an IgA level of 2.19 g/L (0.69 to 3.82 g/L), and an IgM level of 1.61 g/L (0.63 to 2.77 g/L). A urine protein electrophoresis of a 200x concentrated random urine specimen (total protein of 17 mg/dL) revealed a faint protein band in the beta-gamma region, which consisted of free kappa chains. Evaluation of a bone marrow aspirate was remarkable for a mild plasmacytosis (10% to 15% of cellular elements), without any features of plasma cell atypia. The bone marrow biopsy showed a slightly hypercellular marrow (80% cellularity) and a mild plasmacytosis. No organisms were recovered from bacterial, mycobacterial, or fungal cultures of blood or bone marrow. The patient has remained clinically stable since his initial presentation. The abnormal SPEP findings seen on presentation were still present when he was last evaluated in January 1987.

**SPEP and IFE.** SPEPs and IFEs were performed as previously described. Western blot analysis. Western blots to detect HIV serologic reactivity were performed according to the December 1986, Centers for Disease Control (CDC) immunoblot procedure. HIV virions were purified from H9/HTLV-IIIB cell culture supernatants as previously described.

**HPLC-hydroxyapatite paraprotein purification.** Proteins from plasma (50 μL) were precipitated by the addition of Na₂SO₄ to a final concentration of 19% (wt/vol). The precipitate was dialyzed extensively against 0.01 mol/L Na-phosphate, 10 μmol/L CaCl₂, pH 6.5, and applied to an HPLC-hydroxyapatite column for monoclonal antibody purification (MAPS system, Bio-Rad Laboratories, Richmond, CA). Proteins were eluted with a linear gradient of 0.01 to 0.30 mol/L Na-phosphate (volume 30 to 50 mL), 10 μmol/L CaCl₂, at 1 mL/min, with continuous monitoring of the

![Fig 1](https://www.bloodjournal.org)  
**Fig 1.** HPLC-hydroxyapatite column purification of the paraprotein spike. (A) Densitometric scan of column fractions. (B) Anti-HIV reactivity of individual column fractions, as determined by Western blot. The primary reactive protein species include p66\(^*\), p55\(^*\), p53\(^*\), and p24\(^*\). Fraction 24 retained anti-HIV reactivity to a dilution of 1:100. (C) Coomassie Blue-stained SDS-PAGE analysis of column fractions 19 to 31. Molecular weights, expressed in kilodaltons, are as indicated on the ordinates of panels B and C.
effluent at 280 nm. The peak HPLC-hydroxylapatite column fraction of interest was concentrated 80-fold (Amicon CS15 concentrator; Amicon Corp., Danvers, MA), and analyzed by IFE.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was performed as previously described. 14

P24 affinity purification of anti-p24 antibodies. CNBr activated Sepharose 4B beads (Pharmacia) were coupled to 50 μg of purified recombinant HIV p24 (generous gift of C. Debouck, Smith Kline and French Laboratories, Swedeland, PA) at a final concentration of 200 μg p24/mL of beads using the methods published in the Pharmacia package insert. These p24-Sepharose beads were incubated with the HPLC-hydroxylapatite column fraction of interest for 24 hours at 4°C with constant agitation. The supernatant (containing unbound material) was removed, the beads washed ten times with phosphate buffered saline, pH 7.5, and bound antibody eluted with 3 mol/L NaSCN.

Cell lines. The CEM cell line (T cell ALL) and the EBV transformed B cell line, LB2, were grown in RPMI 1640 supplemented with 10% fetal calf serum.

Southern blot analysis for immunoglobulin (JH) gene rearrangement. Mononuclear cells were isolated from an EDTA-anticoagulated bone marrow aspirate, obtained after informed consent according to the University of California, San Francisco Human Subjects Committee guidelines, by Ficoll-hypaque density gradient centrifugation. DNA extraction from 2 × 10^7 RM bone marrow cells, CEM cells and LB2 cells, HindIII and EcoRI double endonuclease digestion, nitrocellulose transfer, and JH probe hybridization were all performed as previously described. 15

RESULTS

Purification of the paraprotein spike. HPLC-hydroxylapatite chromatography was used to purify the paraprotein from the plasma of patient RM. Figure 1A shows that a single major peak of protein eluted from an HPLC column loaded with a 19% (wt/vol) Na2SO4 precipitate of RM plasma. Western blot analysis showed that anti-HIV antibody activity co-migrated with this peak of eluted protein (Fig 1B). The eluted protein peak, fraction 24, contained anti-HIV antibody activity to a final dilution of 1:100 (data not shown). This reactivity was directed primarily at HIV "gag" (p24, p41, p55) and "pol" (p53, p66) gene products. Because the column introduces a dilutional factor of 1,000 when compared with the original starting volume of the sample applied, the corrected reactivity of this fraction was 1:100,000.

SDS-PAGE analysis of the individual column fractions (Fig 1C) revealed the presence of an immunoglobulin heavy chain (Mol wt 50 kd) as well as at least two different sizes of immunoglobulin light chain species (Mol wt 30 to 33 kd).

To verify that we had purified the paraprotein (and not a polyclonal assortment of gamma globulins), we concentrated fraction 24 and analyzed it by IFE (Fig 2). The protein present in this fraction had identical electrophoretic mobility as the paraprotein (Figure 2, lane D).

Analysis of the paraprotein clonality. To address the issue of clonality, we obtained a sample of patient RM's bone marrow mononuclear cells and analyzed the cellular DNA by Southern blot hybridization for evidence of a clonal population of immunoglobulin producing cells. Since 15% of RM bone marrow mononuclear cells were plasma cells, half of them might have been expected to show a clonal immunoglobulin gene rearrangement if they were producing the paraprotein. Figure 3 shows a Southern blot analysis with an immunoglobulin JH gene probe of DNA extracted from RM bone marrow (lane 3) compared with DNA extracted from an established T cell line, CEM (lane 1), and from an EBV

![Image](image.png)
infected and immortalized B cell clone, LB2 (lane 2). The DNA extracted from CEM cells demonstrated the germline configuration of non-rearranged immunoglobulin genes, with hybridizing DNA fragments present at 2.0 and 3.0 kb. The DNA extracted from the B cell clone demonstrated biallelic immunoglobulin rearrangement, with two hybridizable DNA fragments present at 1.8 kb. The DNA extracted from RM's bone marrow showed a germline configuration demonstrating the absence of a clonal population of B cells.

However, the paraprotein, if of clonal origin, could have been produced from a site other than that biopsied. Monoclonal antibodies should recognize only one antigenic epitope. Yet, this patient's purified paraprotein contained antibody reactivity against at least two different HIV gene products ("gag" and "pol"). We affinity purified anti-p24 antibodies from the purified paraprotein (HPLC-hydroxylapatite column fraction 24) to address the issue of whether the paraprotein was recognizing a common epitope shared between "gag" and "pol" gene products or the paraprotein being comprised of a polyclonal group of anti-HIV antibodies. Figure 4 shows that we could separate the anti-HIV "pol" antibody reactivity (Fig 4, lane A) from the anti-HIV "gag" antibody reactivity (Fig 4, lane B), demonstrating the polyclonal nature of this "paraprotein" by the heterogeneity of antigen recognition.

**DISCUSSION**

The observation that transient paraproteinemias were seen in association with infectious diseases suggested that a specific antigenic stimulus was responsible for the production of such immunoglobulins. The purpose of this study was to investigate the antigenic specificity, if any, of a paraprotein seen in an HIV-infected individual with ARC.

We have demonstrated that this particular paraprotein contained anti-HIV activity against HIV "gag" and "pol" gene products, and that this paraprotein alone accounted for most of the anti-HIV antibody reactivity present in this patient's serum. The observation of at least two light chain species in the purified paraprotein fraction, and the antigenic reactivity against multiple HIV gene products, support the conclusion that this paraprotein was not monoclonal in origin. Although we did not detect a clonal B cell population in the patient's bone marrow by immunoglobulin gene analysis, our level of hybridization sensitivity may not have been sufficient to rule out the presence of a minor B cell clone. However, the finding that this paraprotein recognized multiple discrete HIV antigenic determinants coupled with the presence of two light chains rules out the likelihood that this paraprotein was derived from a monoclonal B cell population.

Previous studies have documented polyclonal B cell activation and defective B cell immunoregulation following HIV infection. Hypergamaglobulinemia accompanied by high anti-HIV antibody titers is an early manifestation of HIV infection, suggesting that a vigorous and intact immune response occurs at the time of infection. We would conclude from our study of this patient that a vigorous anti-HIV antibody response was still present, but atypical in that the majority of his anti-HIV antibodies were of a similar charge, such that they migrated in a tight band on SPEP, giving the appearance of a paraprotein.

A paraprotein is defined by current laboratory methods as an abnormal immunoglobulin that migrates as a single band and is composed of a single species of heavy and light chains. The restricted expression of the immunoglobulin chains had led to the prevailing concept that paraproteins are the products of single clones of B cells. Paraproteins have been previously observed in AIDS and ARC patients (ie, in those patients with advanced disease attributable to HIV); the definition of monoclonality of these paraproteins was made by immunologic methods, in which the paraprotein(s) were shown to be of one class (usually IgG kappa) only. The data presented in this study showed, however, that a paraprotein that was comprised predominantly of IgG kappa was not of monoclonal origin. Sophisticated laboratory studies, usually not available to the routine clinical laboratory, were necessary to disprove monoclonality.

Because the finding of paraproteins in HIV-infected individuals is not infrequent, we recommend that the full laboratory evaluation of such patients be stratified—if a paraprotein is seen in the setting of a polyclonal hypergammaglobulinemia in an HIV-infected patient, then further clinical and laboratory evaluation for a plasma cell malignancy is not necessary. However, if other clinical signs suggestive of a pathologic monoclonal gammopathy (ie, hypercalcemia, lytic bone lesions, atypical plasma cells in the bone marrow) are present, then a full evaluation is warranted.

In other transiently expressed paraproteins seen in association with various infectious diseases, the paraproteins disappeared with resolution of the disease. However, HIV infection is a chronic life-long infection; thus, we would expect this patient's paraprotein to be continually expressed for the remainder of his lifetime. In fact, we have observed the continued expression of this paraprotein for at least 1.5 years after the initial observation. The chronicity of HIV infection would also explain the continued observance of oligoclonal and monoclonal paraproteins in other ARC and AIDS patients.

![Affinity purification of anti-p24 antibodies and Western blot analysis of antibody reactivity](image)
PARAPROTEIN-ASSOCIATED HIGH-TITER ANTI-HIV ANTIBODIES

It is intriguing to speculate on the origin of high grade B cell lymphomas in the AIDS population in light of this study. One study demonstrated the presence of multiple monoclonal B cell populations in patients with lymphadenopathy syndrome (LAS) and AIDS. However, only in AIDS patients with B cell lymphoma was a rearranged c-myc gene present. This suggested that although HIV infection was instrumental in the expansion of multiple B cell clones, another event (resulting in c-myc rearrangement) was necessary for the ultimate development of lymphoma, presumably from one of the expanded B cell clones already present. Perhaps HIV-infected patients who have paraproteins are already at the stage of expanded multiple B cell clones, and that another event will trigger the selective expansion of one of these clones to result in lymphoma. We are currently following up our patient and other similarly HIV infected patients with paraproteins to see if there is an increased incidence of B cell lymphomas within this population.

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