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Recombinant Polypeptide from the Endonuclease Region of the Acquired Immune Deficiency Syndrome Retrovirus Polymerase (*pol*) Gene Detects Serum Antibodies in Most Infected Individuals

KATHELYN S. STEIMER,* KEITH W. HIGGINS, MAUREEN A. POWERS, JAMES C. STEPHANS, ALEXANDER GYENES, CARLOS GEORGE-NASCIMENTO, PAUL A. LUCIW, PHILIP J. BARR, ROBERT A. HALLEWELL, AND RAY SANCHEZ-PESCADOR

Chiron Corporation, Emeryville, California 94608

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Sera from the majority of individuals that were positive in an enzyme-linked immunosorbent assay (ELISA) for antibodies to acquired immune deficiency syndrome (AIDS)-associated retrovirus (ARV), an isolate of the retrovirus identified as the etiologic agent of AIDS, were found to react with a 31,000-dalton protein (p31) in virus Western blot assays. To determine if this 31,000-dalton immunoreactive species originated from the putative endonuclease region of the polymerase (*pol*) gene of ARV, we cloned this portion of *pol* into bacterial expression vectors for direct expression and for expression as a fusion protein with human superoxide dismutase. Transformants from both constructions expressed immunoreactive protein detected in immunoblots with an AIDS patient's serum. Extracts from transformants expressing these sequences competed with the binding of antibodies from AIDS patients' sera to the 31,000-dalton protein in virus immunoblots, confirming that viral p31 originated from the endonuclease domain of the ARV polymerase gene. The superoxide dismutase-p31 fusion protein was purified, and an ELISA for detecting antibodies to p31 was developed. The majority (95%) of serum samples obtained from individuals seropositive in the virus ELISA were also positive in the p31 antibody ELISA.

A novel human retrovirus has been implicated as the causative agent of acquired immune deficiency syndrome (AIDS). Three independent isolates of this virus have been molecularly cloned (1, 9, 19) and sequenced (23, 25, 28, 33). These isolates, referred to as lymphoadenopathy-associated virus (2), human T-cell lymphotropic virus type III (24), and AIDS-associated retrovirus (ARV) (18) by the laboratories in which they were isolated, are very closely related. The AIDS retrovirus has been isolated from numerous patients with AIDS and related disorders (2, 7, 18, 24, 27) from asymptomatic members of groups at high risk for AIDS (27), and from patients without other risk factors who have contracted AIDS after receiving blood transfusions (6). Several techniques have been used to detect antibodies to the AIDS retrovirus, including immunoblotting (29), enzyme-linked immunosorbent assay (ELISA) (3, 35), radioimmunoprecipitation (15), and immunofluorescence (14). Most patients with AIDS or diseases associated with this syndrome (AIDS-related condition [ARC]) have serum antibodies to the virus structural proteins (15, 29). In addition, a high proportion of clinically healthy members of high-risk groups such as male homosexuals (8, 17, 26), hemophiliacs (15), and intravenous drug users (3) are also seropositive.

In studying the immune response to individual viral proteins by immunoblot analysis on electrophoresed ARV proteins, we observed that a high proportion of seropositive individuals had serum antibodies that reacted with a viral polypeptide of approximately 31 kilodaltons (kDa). This polypeptide did not correspond to the expected molecular mass of any of the previously identified viral structural proteins (15, 28, 29). Homology comparisons of ARV-2 to other retroviruses support the model that the ARV *pol* gene encodes a precursor polypeptide that is processed to produce the following three proteins: protease, reverse transcriptase, and endonuclease (28). For Rous sarcoma virus, the proteolytic processing site that generates a 32-kDa endonuclease has been identified. Some amino acid sequence homology between Rous sarcoma virus and ARV-2 has been noted near the cleavage site that generates the Rous sarcoma virus endonuclease (11). Thus, ARV-2 is predicted to encode a 31-kDa polypeptide from the carboxy-terminal domain of *pol*. We report here the expression in *Escherichia coli* of the region of the ARV-2 polymerase gene corresponding to the endonuclease domain and show that the viral p31 protein visualized in virus immunoblots is derived from this region of the viral genome. Recombinant p31, produced as a fusion protein with human superoxide dismutase (SOD), was purified, and a specific ELISA for p31 antibodies was developed. In agreement with the results of our virus immunoblot assays, we found that most, but not all, individuals that were seropositive for virus had antibodies specific for the p31 antigen.

MATERIALS AND METHODS

Virus and cells. The ARV-2 isolate of ARV was provided by J. A. Levy, University of California, San Francisco. Virus was propagated in HUT-78 cells (obtained from the American Type Culture Collection, Rockville, Md.), concentrated, and purified by sucrose density gradient purification as described previously (28).

Human sera. Serum EW5111 (reference serum from an AIDS patient) was obtained from P. Feorino, Centers for Disease Control, Atlanta, Ga. Serum 0036 from an AIDS patient was purchased from Trimar, Reseda, Calif. Pooled normal human serum was from Medical Specialties Laboratories, Boston, Mass. Gary E. Tegtmeier of the Community Blood Center of Greater Kansas City, Kansas City, Mo., provided normal sera from consecutive blood donors. The 85

* Corresponding author.

seropositive samples were selected from a panel of sera provided by the AIDS Specimen Bank, University of California, San Francisco.

Expression of p31 in *E. coli*. A 1,848-base-pair DNA fragment produced by digestion with *Kpn*I and *Sac*I encodes a portion of the putative endonuclease region of the *pol* gene of ARV-2 (28). This DNA fragment was cloned into bacteriophage M13 mp19 by standard techniques (20). Mutagenesis of this clone was performed as previously described (36) with the following synthetic oligonucleotide primers: 5'-TTAAAATCACTTGCCATGGCTCTCCAATT ACTG-3' and 5'-GGTGTTTTTACTAAAGAATTCCGTCG ACTAATCCTCATCC-3'. The mutagenic primers were designed to introduce new *Nco*I, *Eco*RI, and *Sal*I sites for subsequent modification. All oligonucleotides were synthesized by the phosphoramidite method with Applied Biosytems 380A DNA synthesizers. The modified gene was excised from the replicative form of bacteriophage M13 and cloned as an *Nco*I-*Eco*RI fragment into plasmid pTAC7 (10). Plasmids were grown in *E. coli* RR1ΔM15 (20, 21), and recombinants were selected. The modified gene was extended by digesting the above plasmids with *Pvu*II and *Nco*I and subsequently inserting synthetic linkers (see Fig. 2a). After filling the ends with the Klenow fragment of DNA polymerase I and ligating with T4 DNA ligase, we used the resulting plasmids to transform *E. coli* RR1ΔM15 and selected recombinants for an insert of the predicted size and the appropriate restriction sites (20). After screening several transformants for expression of p31 antigen (see below), we selected the highest producer, designated pTp31.2, for further evaluation.

Similarly, the *Nco*I-*Sal*I fragment from the mutagenized M13 phage was cloned into the vector pSODCF2, a vector containing the human SOD gene under the control of the *tac*I promoter to produce plasmid pTSp31 containing a fusion with the SOD gene in the same translation frame. Plasmid pSODCF2 was derived from pSODCF1, which is a derivative of pSODX16 (10), in which 67 base pairs of DNA from the *Sau*3A site at Gly 150 of SOD to the *Bam*HI site in the polylinker were removed, regenerating a *Bam*HI site at Gly 150. A synthetic polylinker of sequence

*Bam*HI *Nco*I *Sma*I stop codons

5'-GATCGCCATGGGTACCCGGGTCGACTAAATGACTAGAATT-3' (coding strand)

3'-CGGTACCCATGGGCCCAGCTGATTTACTGATCTTAA-5'

*Sal*I

was inserted between the *Bam*HI and *Eco*RI sites of pSODCF1 to make pSODCF2.

Bacterial extracts for gel analysis and competition experiments. (i) Growth and induction of bacteria. Bacterial transformants containing either the vector alone (pTAC7), the vector with the p31 DNA insert (pTp31.2), or the vector with the SOD-p31 DNA insert (pTSp31.8) were grown in L broth with 0.02% ampicillin to an optical density at 650 nm of 0.5. The cultures were induced by adding 2 mM isopropyl-β-D-thiogalactopyranoside (Sigma Chemical Co.) for 3 h. Uninduced cultures were grown under identical conditions without addition of isopropyl-β-D-thiogalactopyranoside.

(ii) Bacterial extracts for SDS-polyacrylamide gel analysis. Bacteria from 1 ml of culture were pelleted and suspended in 200 μl of electrophoresis sample buffer. The cells were disrupted by three cycles of freezing and thawing. The resulting extract was boiled for 10 min before sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

(iii) Bacterial lysates for competition experiments. Bacteria from 5 ml of culture were pelleted and suspended in 1 ml of 50 mM Tris (pH 8.0)–0.5 mM EDTA–1 mg of lysozyme (Sigma) per ml, and the suspension was incubated for 15 min on ice. At the end of incubation, the solution was adjusted to 0.4 M NaCl–5 mM $MgCl_2$–0.5% Nonidet P-40 and mixed by gently inverting the tube. DNase I was added to the solution (100 μg/ml; Sigma), and the mixture was incubated at 37°C for 30 min. The tube was swirled gently every 5 min. At the end of incubation, the extract was centrifuged at 12,000 × *g* for 5 min, and the supernatant was used immediately or stored frozen at −20°C.

Purification of SOD-p31. Ten liters of culture of *E. coli* with the pTSp31.8 plasmid was grown and induced with isopropyl-β-D-thiogalactopyranoside as described above. The pelleted bacteria were mixed with 3 volumes of lysis buffer (20 mM Tris, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride [pH 8.0]). The cells were disrupted with glass beads in a Dyno-Mill cell mill (Willy A. Bachofen Manufacturers, Basel, Switzerland). The SOD-p31 protein was present in the insoluble fraction after cell disruption. The cell lysate was centrifuged at 39,000 × *g* for 30 min, the supernatant was discarded, and the pellet was suspended in lysis buffer containing 0.2% SDS. The suspension was centrifuged at 39,000 × *g* for 30 min, and the resulting insoluble pellet containing the SOD-p31 protein was solubilized in 2.3% SDS–5% β-mercaptoethanol–67.5 mM Tris (pH 7.0). The SOD-p31 was fractionated by gel filtration with an ACA-34 column (LKB Instruments, Inc.) equilibrated with phosphate-buffered saline (PBS; pH 7.4) containing 0.1% SDS. Fractions were tested for SOD-p31 by immunoblot analysis with AIDS serum with antibodies to p31. Peak fractions, approximately 95% pure SOD-p31 (as revealed by SDS-polyacrylamide gel electrophoresis), were pooled, concentrated, dialyzed, and used to coat microtiter plates for the ELISA.

Immunoblot procedure. All samples were electrophoresed on standard Laemmli discontinuous SDS-polyacrylamide gels under reducing conditions (16) with a Bio-Rad Laboratories mini gel apparatus. A 0.5-cm well for prestained molecular mass standards and an 8-cm well for the sample were prepared in the stacking gel when gels were being prepared for making immunoblot strips. When multiple samples were being evaluated, 0.5-cm wells were prepared in the stacking gel. The volumes of sample loaded were 5 μl in 0.5-cm wells and 100 μl in 8-cm wells. Following electrophoresis, proteins were transferred to nitrocellulose filters by the procedure of Towbin et al. (32). Blots with 8-cm sample wells were cut into 2-mm strips, and each strip was processed separately. The blots were processed with Carnation nonfat dry milk (13) in the diluents by the following procedure. Filters were pretreated for 30 min with 5% nonfat dry milk in PBS, the pretreating solution was removed, and then diluted serum was added in 5% nonfat dry milk in PBS. The blots were incubated for 1 h at 37°C and then washed three times with PBS. Horseradish peroxidase-conjugated

goat antiserum to human immunoglobulin G (Cappel Laboratories; no. 3201-0081) diluted 1/200 in 5% nonfat dry milk in PBS was added for 1 h. The conjugate solution was removed, and the blots were washed three times in 0.1% Triton X-100 in PBS and developed with HRP color development reagent (containing 4-chloro-1-napthol; Bio-Rad).

ELISA. Human sera were assayed for antibodies to AIDS retroviruses by a modification of the procedure described previously (3, 30, 35). For this assay, gradient-purified ARV-2 was used as a source of antigen. Microtiter plates were coated with 5 μg of disrupted virus per ml, and all sera were diluted 1/100 for assay. Horseradish peroxidase-conjugated goat antiserum to human immunoglobulin (Cappel; no. 3201-0081) diluted 1/4,000 was used as the second-stage antibody, and 150 μg of 2,2′-azino-di-(3-ethylbenzylthiazolinesulfonic acid) per ml–0.001% H_2O_2–0.1 M citrate [pH 4.0] was used as the substrate.

We assayed human sera for antibodies to p31 by coating microtiter plates (100 μl per well) with 2 μg of purified SOD-p31 per ml in borate buffer (0.05 M, pH 9.0). Sera were tested for p31 antibodies at a dilution of 1/100 unless indicated otherwise in the figures or table legends. Included in the serum diluent was a 1/100 dilution of an extract from an induced culture of an *E. coli* strain transformed with pTAC-7, the vector without the p31 DNA insert. This was essential to absorb antibodies to *E. coli* proteins that reacted with minor contaminants that were present in the purified SOD-p31 preparation. The horseradish peroxidase conjugate and substrate were as described above.

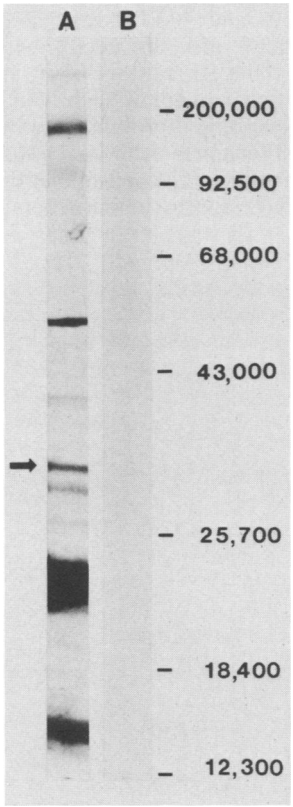

FIG. 1. Virus immunoblot assay. Strips of immunoblots of ARV-2 virus were reacted with either a 1/50 dilution of serum 0036 from an AIDS patient (lane A) or a 1/50 dilution of pooled normal human sera (lane B). Molecular mass standards were 200,000 (myosin H chain), 97,400 (phosphorylase B), 68,000 (bovine serum albumin), 43,500 (ovalbumin), 25,700 (α-chymotrypsinogen), 18,400 (β-lactoglobulin), and 12,300 daltons (cytochrome *c*). The arrow indicates the 31-kDa viral polypeptide.

TABLE 1. Frequency of reactivity of AIDS virus-seropositive samples with the 31-kDa polypeptide in virus immunoblot assays[a]

Group	No. tested	No. with antibodies to viral p31	% Virus-seropositive individuals with p31 antibodies
Contacts[b]	21	20	95
ARC	26	23	88
AIDS	38	31	82

[a] These sera all scored positive at 1/100 dilution in an ELISA with disrupted, gradient-purified ARV-2 virus as the antigen.
[b] Asymptomatic, seropositive sexual contacts of patients with AIDS or ARC.

RESULTS

AIDS sera react with a 31-kDa viral polypeptide. In characterizing the humoral immune response to individual viral antigens of patients infected with the AIDS retrovirus, we observed that most seropositive individuals have antibodies that react with a 31-kDa polypeptide present in our virus preparations. Figure 1 shows the results of a typical immunoblot assay with serum from an AIDS patient with a high antibody titer to the virus (lane A). Prominent reactivity of this serum was seen with the major *gag* (core) protein p25, the polyprotein precursor of the *gag* proteins (p53), the series of bands in the 41- to 43-kDa region of the gel which are thought to represent the C-terminal portion of the envelope protein of the virus (gp41; 15, 29), the 120-kDa envelope protein (gp120; 15, 22, 29), and the 31-kDa band (arrow).

The frequency of reactivity on virus Western blots of 85 virus seropositive samples with the 31-kDa polypeptide is presented in Table 1. These sera were obtained from patients with AIDS, patients with ARC, or clinically healthy sexual contacts of AIDS or ARC patients (contacts). Sera were categorized as virus seropositive if they scored positive in an indirect ELISA for viral antibodies with disrupted, gradient-purified ARV as the antigen. Of these 85 sera, 74 (87%) reacted with the 31-kDa species. When the results were tabulated according to diagnosis, 95% of the contacts, 88% of the ARC patients, and 82% of the AIDS patients had serum antibodies to this protein.

Subsequent to our identification of p31 as a potentially important viral antigen, there was a report that many AIDS sera reacted with a 15-kDa polypeptide produced by recombinant DNA techniques that originated from the carboxyl terminus of the human T-cell lymphotropic virus type III polymerase gene (4). Furthermore, this polypeptide was able to absorb out antibodies in an AIDS patient's serum that reacted with a 31-kDa protein in virus Western blot assays. We thus set out to clone and express the endonuclease region of the polymerase gene to determine if the viral p31 protein with which our seropositive samples were reacting was from this region of the viral genome.

Cloning and expression of p31 in *E. coli*. The polymerase (*pol*) gene encodes a 1,003-amino-acid polyprotein which, by analogy with other retroviruses, would give rise to protease, reverse transcriptase, and endonuclease activities (11, 28, 34). For expression of the putative endonuclease region, we modified the DNA coding for the carboxyl region of the *pol*

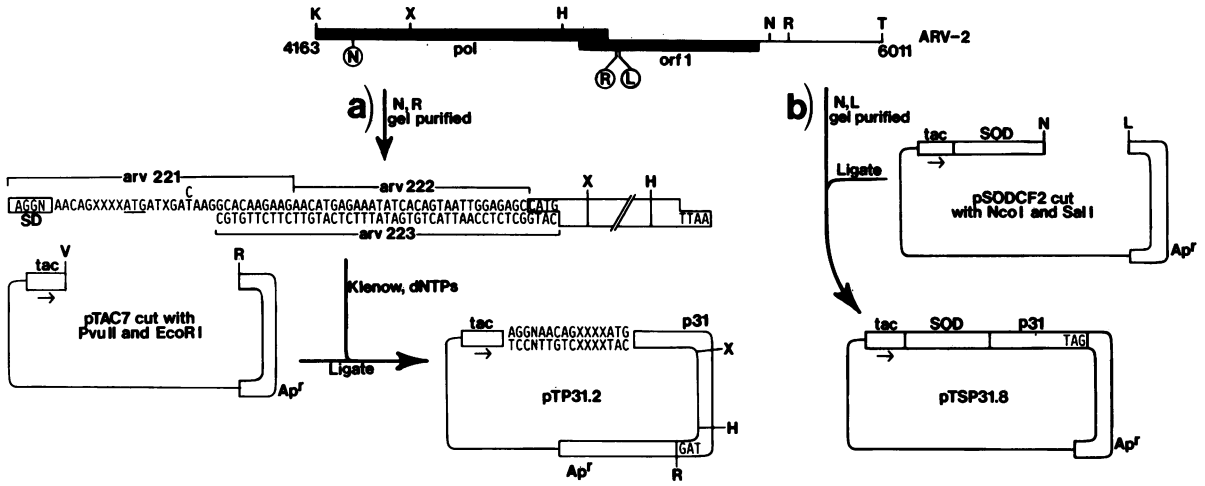

FIG. 2. Construction of p31 expression vectors. (a) An *Nco*I (N)-*Eco*RI (R) fragment of ARV-2 was extended with synthetic linkers, as described in the text, and cloned into pTAC7 cut with *Pvu*II (V) and *Eco*RI. The first ATG is underlined, and the Shine-Dalgarno (SD) sequence (32) is boxed in. Nucleotide N refers to any one G, A, T, or C. Nucleotide X corresponds to C, A, or T. (b) An *Nco*I-*Sal*I (L) fragment of ARV-2 was cloned between the N and L sites of pSODCF2 for expression of a fused protein, SOD-p31. K, X, H, and T correspond to *Kpn*I, *Xba*I, *Hin*dIII, and *Sst*I, respectively. Encircled restriction sites indicate sites introduced by in vitro mutagenesis.

gene by site-specific mutagenesis. A unique *Nco*I site was introduced at amino acid position 736 (28) by means of silent third-position changes in codons. In addition, *Eco*RI and *Sal*I sites were introduced 3′ to the *pol* gene termination codon to allow facile removal of this region of the gene and cloning into expression vectors. To obtain high-level transcription of the p31 gene, we used the *tac*I promoter (5). To optimize translation initiation from the p31 mRNA, we used randomly generated synthetic ribosome binding sites as described previously (10, 12) (Fig. 2a). Note that secondary structure predictions (10, 37) required that we randomize the Shine-Dalgarno sequences (31) in this construction.

In addition to direct expression of p31, the *Nco*I-*Sal*I DNA fragment described above, containing the p31 gene, was fused to the carboxyl end of the gene for human SOD. High-level expression of SOD in *E. coli* with a similar vector has been described previously (10). Furthermore, SOD fusions with heterologous proteins have been shown to produce hybrid proteins of high stability in both bacteria and yeast (P. Tekamp-Olson and L. Cousens, personal communication). The construction for production of the SOD-p31 hybrid protein under control of the *tac*I promoter is shown in Fig. 2b, and the structure of pSODCF2 is given in Materials and Methods.

Reactivity of antibodies in an AIDS patient's serum with recombinant p31. Extracts prepared from induced cultures of bacterial transformants containing either the vector alone (pTAC7), the vector with the p31 DNA insert (pTp31.2), or the SOD-p31 DNA insert (pTSp31.8) were electrophoresed on SDS-polyacrylamide gels (Fig. 3). Gels were either stained with Coomassie brilliant blue (panel 1) or transferred to nitrocellulose by electroblotting and reacted with serum from an AIDS patient (panel 2) that we had already determined reacted strongly with viral p31. An immunoreactive band at 30 kDa, precisely the molecular mass expected from the coding capacity of the insert, and several lower-molecular-mass species, were observed in immunoblots of an extract from the induced pTp31.2 transformant (lane C). A prominent reactive band at approximately 48 kDa was present in extracts from bacteria transformed with the SOD-p31 gene fusion (lane A); this was the molecular mass expected for a fusion protein originating from the sum of the SOD and p31 sequences that were inserted into the transforming vector. Similarly immunoreactive species were not present in the 30- and 40-kDa regions of extracts from bacteria transformed with the vector without the p31 or SOD-p31 inserts (lane B). Furthermore, immunoblots with nonimmune sera (from a pool of healthy individuals) did not react with either expressed protein (data not shown).

Recombinant p31 competes with viral p31 for binding of p31 antibodies in human sera. To confirm that the 31-kDa protein that was visualized on virus immunoblots corresponded to

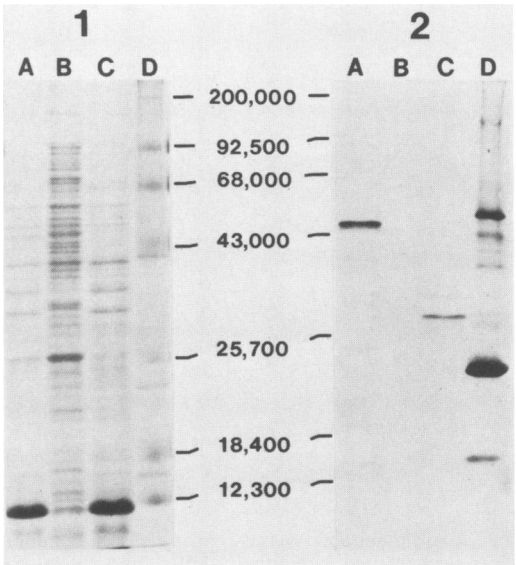

FIG. 3. Detection of recombinant p31 in lysates from *E. coli* transformants by immunoblot analysis. Panels: 1, Coomassie blue-stained gel; 2, immunoblot of a 1/100 dilution of an AIDS patient's serum (EW5111). Lanes: A, pTSp31.8; B, pTAC.7; C, pTp31.2; D, virus. The molecular mass standards are described in the legend to Fig. 1.

the endonuclease region of the viral polymerase gene expressed in *E. coli*, we made cell extracts of our transformants and used them in competition experiments. Virus (ARV-2) was disrupted and electrophoresed on SDS-polyacrylamide gels, the gels were electroblotted, and the nitrocellulose blot was cut into strips. Each strip was incubated with diluted serum in the presence or absence of bacterial lysates (Fig. 4). The reactivity of EW5111 (lane A), a high-titer serum that reacts with most of the viral antigens seen in Western blots, with viral p31 was completely eliminated when it was preincubated with an extract from induced pTp31.2 transformants (lane C). In contrast, reactivity with the other viral structural proteins was unaffected. In addition, there was no competition with viral p31 reactivity when EW5111 was preincubated with an extract from induced pTAC7 transformants (lane B), the strain transformed with the vector lacking the p31 insert. A more striking illustration of this finding is shown in the right half of the figure, where the results with serum 0075, which reacted exclusively with p31 in virus immunoblots, are presented. Identical results to those shown in Fig. 4 were obtained in competition experiments with lysates from bacteria expressing the SOD-p31 fusion protein (data not shown).

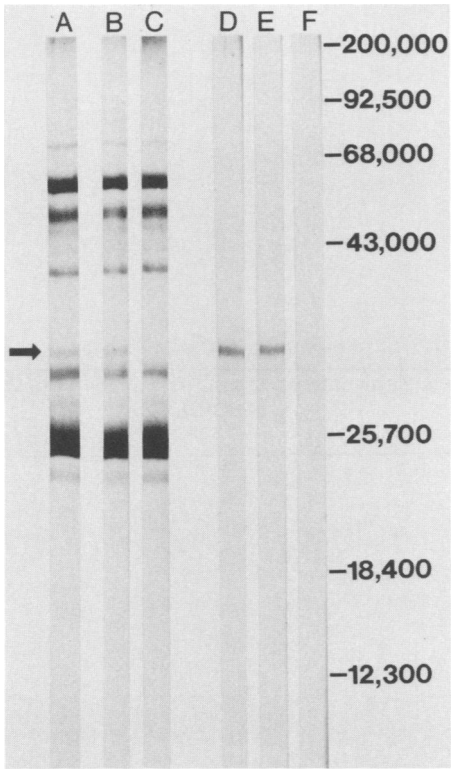

FIG. 4. Competition of extracts from *E. coli* expressing the putative endonuclease domain of the ARV polymerase gene with the binding of antibodies in AIDS sera to viral p31. Virus immunoblots were incubated with serum EW5111 (lanes A to C) and (A) no addition; (B) lysate from *E. coli* transformed with pTAC7, the vector without the p31 insert; or (C) lysate from *E. coli* transformed with pTp31.2, the vector containing the p31 insert. Virus immunoblots were incubated with serum 0075 (lanes D to F) and (D) no addition; (E) pTAC7 lysate; or (F) pTp31.2 lysate. Both sera were diluted 1/100 in the assay, and the extracts were used at a 1/100 dilution. Molecular mass standards are described in the legend to Fig. 1. The arrow indicates the 31-kDa band.

TABLE 2. Comparison of Western blot results of various virus-seropositive samples in viral p31, recombinant p31 expressed directly in *E. coli*, and recombinant p31 expressed in *E. coli* as a fusion protein with human SOD[a]

Serum no.	Reactivity with:		
	Viral p31	Recombinant p31	Recombinant SOD-p31
4607	+	+	+
4608	+	+	+
4620	+	+	+
4625	+	+	+
4626	+	+	+
4642	−	−	−
4643	+	+	+
4646	+	+	+
4659	−	−	−
0036	+	+	+
NHS[b]	−	−	−

[a] Strips from immunoblots of electrophoresed virus, pTp31.2 extracts, and pTSp31.8 extracts were reacted with a 1/100 dilution of each serum.
[b] NHS, Pooled normal human sera obtained from Medical Specialties Laboratories (see Materials and Methods).

Comparison of Western blot assays on viral and recombinant p31. We selected a panel of 10 sera that scored positive in our ELISA for viral antibodies with disrupted virus as the antigen to compare the immunoreactivity of viral p31 with those of the two recombinant proteins. This panel included eight sera that were positive and two sera that were negative for antibodies to viral p31 in virus immunoblots. Lysates of pTp31.2 and pTSp31.8 transformants were electrophoresed and electroblotted, and strips of the blots were reacted with individual serum samples (Table 2). The eight sera that were positive on viral p31 also reacted with the 30- and 48-kDa bands in lysates of bacterial cells containing pTp31.2 and pTSp31.8, respectively. The two sera that were negative on

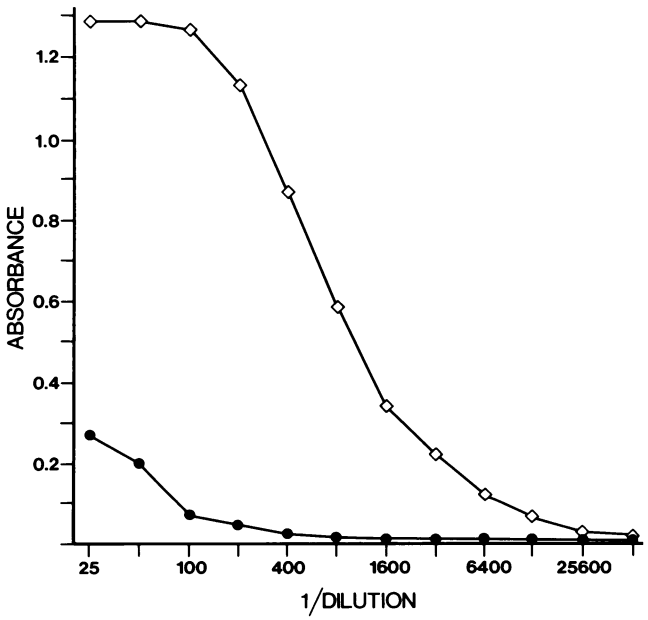

FIG. 5. Titration of antibodies to p31 by ELISA. Pooled normal human sera (●) and serum from an AIDS patient (serum 0036) (◇) were diluted 1/25 and by serial twofold dilutions thereafter. Each point of the graph represents the average of duplicate assay wells.

viral p31 were also negative on both of the recombinant proteins. Pooled normal human serum was negative in all three Western blot assays.

ELISA for p31 antibodies in human sera. The SOD-p31 fusion protein was purified and used as a source of antigen for an ELISA for testing sera for antibodies to p31. We chose to purify the fusion protein rather than the directly expressed protein because we had determined by a competition assay (Fig. 4), by using serial dilutions of extracts of pTp31.2 and pTS31.8 transformants, that the level of expression of p31 antigen was approximately fivefold greater in bacteria transformed with the latter plasmid (data not shown). Prior to deciding to pursue the fusion protein as a source of antigen for our assay, we screened sera from 300 random blood donors and a panel of 100 sera obtained from high-risk, AIDS, and ARC patients for antibodies to purified human SOD in an ELISA. None of these sera scored positive (data not shown). Figure 5 shows titration curves of an AIDS patient's serum and pooled normal human sera on microtiter plates coated with 2 μg of purified SOD-p31 fusion protein per ml. The diluent included an extract from *E. coli* (pTAC7). This extract was necessary to absorb out antibodies in human sera that reacted with *E. coli* proteins that were minor contaminants in the SOD-p31 preparation. As seen in this figure, normal serum did not react with SOD-p31. However, positive serum reacted strongly, with the midpoint of the titration curve occurring at a serum dilution of 1/1,250.

A panel of sera from 100 consecutive blood donors that were all seronegative in the virus ELISA was tested in the p31 ELISA. These sera all scored very low in the assay (Fig. 6a). The average ELISA result for these sera when they were assayed at a 1/100 dilution was 0.026 with a range of 0.001 to 0.117 and a standard deviation of 0.012. The results obtained with the 85 virus-seropositive samples (Table 1) are presented in Fig. 6b. The ELISA results with sera that did not react with the p31 band in the virus Western blot assay are indicated by shading. In general, sera that were positive in virus Western blots for p31 antibodies were clearly positive in the p31 antibody ELISA. However, there were three sera that did not react with p31 in virus immunoblots that were clearly positive in the p31 ELISA.

We considered three possible explanations for viral p31 immunoblot-negative sera scoring positive in the p31 ELISA. (i) The ELISA was more sensitive than the virus immunoblot assay for detecting antibodies to p31; (ii) these sera scored positive in the ELISA owing to immunological reactivity with *E. coli* proteins contaminating the SOD-p31 preparation that were not absorbed out by the bacterial lysate in the antibody diluent; or (iii) these sera scored positive in the ELISA because they contained antibodies to the SOD portion of the fusion protein. To clarify this, we tested these three sera on blots of directly expressed p31 by using the pTP31.2 lysate, which should contain a much higher concentration of p31 antigen than virus. All three of these sera reacted with the p31 species in this assay (data not shown). Thus, these sera scored positive in the ELISA because of its greater sensitivity than virus immunoblots for detecting p31 antibodies, not because of reaction with *E. coli* contaminants or SOD. We also tested the remaining eight viral p31 Western blot-negative sera in immunoblot assays of pTp31.2 lysates. The five sera scoring lowest in the ELISA (Fig. 6b) failed to react with p31 in this assay. However, the three remaining sera, scoring between 0.15 and 0.3 in the ELISA, reacted in immunoblots with directly expressed p31 (data not shown). Thus, we concluded that, of this panel of 85 seropositive samples, 80 (95%) had detectable antibodies to the p31 antigen.

DISCUSSION

We expressed the sequences encoding the predicted 31-kDa endonuclease protein of the ARV-2 isolate of the AIDS retrovirus in *E. coli*. This protein was expressed both directly and as a gene fusion with the sequence coding for human SOD. Competition experiments demonstrated that the 31-kDa immunoreactive species that was visualized in our virus Western blot assays originated from this region of the viral genome. The immunoreactivities of p31 expressed directly and as a fusion protein with human SOD were similar and agreed with the results of immunoblot assays of natural p31 in virus. The SOD-p31 fusion protein was purified and used as the antigen in an ELISA for p31 antibodies. Sera from 100 blood donors, all shown to be seronegative for viral antibodies in a conventional virus ELISA, failed to react in the p31 ELISA. However, 95% of serum samples obtained from virus-seropositive individuals were positive in this assay.

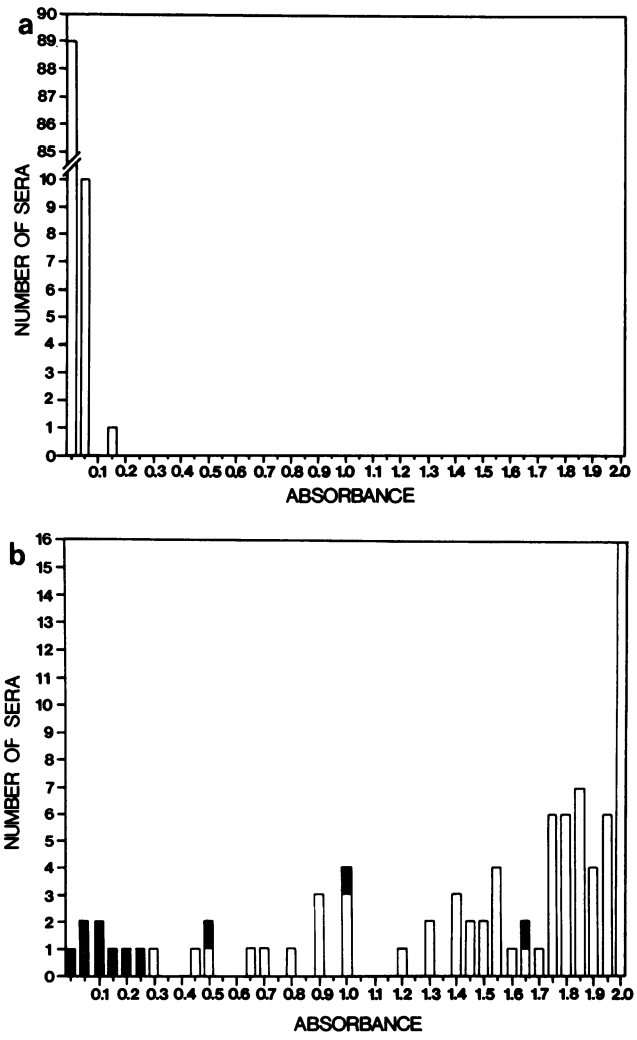

FIG. 6. ELISA survey for p31 antibodies. Panels: a, random (normal) blood donors; b, virus-seropositive individuals. Results with sera that scored negative for p31 antibodies in the virus immunoblot assays (panel b) are indicated by shading.

Our results confirm and extend those of Chang et al. (4). They produced a 15-kDa polypeptide in *E. coli* corresponding to approximately the C-terminal half of the endonuclease region of the polymerase gene of human T-cell lymphotropic virus type III. Twenty AIDS sera were tested by immunoblot assay on this polypeptide, and all were positive. They proposed that testing for antibodies to this 15-kDa peptide could be a means for screening for human T-cell lymphotropic virus type III infection. We have found that most seropositive individuals, including ARC patients, clinically healthy sexual contacts of AIDS patients, and patients with frank AIDS, have serum antibodies to the 31-kDa protein. However, we identified virus-seropositive individuals that clearly lacked antibodies to this antigen. Thus, the use of p31 as the sole antigen in a screening assay would not be adequate.

In natural infections with retroviruses, the host generally makes antibodies to the products of the *gag* or *env* gene or both. Antibody responses to the products of the *pol* gene during infection have not been described. The results presented here, together with those of Chang et al. (4), demonstrate that the majority of individuals infected with the AIDS retrovirus have readily detectable levels of antibodies to the protein derived from the endonuclease region of the *pol* gene. The significance of immune responses in humans to this protein remains to be established. Also, the immunogenicity of the products of the other two domains of the *pol* gene, protease and reverse transcriptase, have not been evaluated. Expression vectors similar to those described in this report may be used to produce polypeptides representing both protease and reverse transcriptase to determine if these proteins are also immunogenic in infected individuals.

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