

Human Plasmatic Apolipoprotein H Binds Human Immunodeficiency Virus Type 1 and Type 2 Proteins

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ABSTRACT

Apolipoprotein H (apo H), isolated from human plasma albumin solution, was shown to capture HIV-1-related antigens from antigen-positive sera (HIV-1 AG⁺) of AIDS patients, by using HIV-1-specific polyclonal antibodies. In an enzyme-linked immunosorbent assay and ligand blot and dot assays, apo H was able to bind recombinant retroviral HIV antigens, especially Gag proteins p18 of HIV-1, p26 of HIV-2, and Env gp160 of HIV-1. Binding was shown to be pH and NaCl dependent, with an optimum at acidic pH and low ionic strength. Specificity was demonstrated by saturation of this binding and inhibition either by homologous competition or by specific antisera. Binding was also observed in cell line-harvested viral blotted proteins. The mechanism of this apo H-polyspecific binding is discussed in relation to conformational changes due to the influence of lipids or detergents.

INTRODUCTION

A NUMBER OF STUDIES have reported isolation of the human immunodeficiency virus (HIV) from biological fluids such as plasma and serum.^{1,2} Variations in culture conditions, such as delaying blood processing time, can affect the culture success rate for HIV isolated from plasma.^{3,4} This suggests the presence of antiviral factors in the plasma and the existence of physical interactions between viral particles and plasma components. Of these latter components, the complement system, fibronectin, and mannose-binding protein have been studied extensively (for review see Ref. 5). Moreover, apolipoprotein A-1 (Apo A-1), the main protein component of high-density lipoproteins (HDLs), interacts with N-terminal fusogenic domains of simian immunodeficiency virus (SIV) gp32 and HIV gp41 and inhibits viral infectivity and syncytium formation.^{6,7}

Human serum albumin (HSA) polymers are known to bind hepatitis B virus (HBV).⁸ We purified a 50-kDa protein in order to investigate a possible component responsible for this binding activity in HSA solutions. N-Terminal amino acid sequencing allowed us to identify this protein as apolipoprotein H (apo H).⁹ A French patent claimed the binding of apo H to various viral components.¹⁰ Notably, in HBV-infected sera, apo

H was able to bind a factor detected by monoclonal anti-HBsAg antibodies. Others have since demonstrated direct binding between apo H and HBsAg.¹¹

Apo H, also known as β_2 -glycoprotein I (β_2 -GPI), was originally identified in plasma as a glycoprotein that is soluble in perchloric acid.^{12,13} Apo H (50-kDa molecular mass and about 200- μ g/ml plasmatic concentration) was also reported to be among the apolipoproteins found on the HDL.¹⁴ Its amino acid sequence showed an unusual composition for a plasma protein, with 6.2% cysteine and 8.3% proline.¹⁵ This protein is composed of 5 segments of 60–80 amino acid residues, each containing the consensus sequence of the complement control protein (CCP) module, which implies that apo H is a member of the CCP superfamily.¹⁶

About 30% of serum apo H is associated with the lipoprotein fraction.¹⁴ Apo H binds to negatively charged substances such as anionic phospholipids, as well as macromolecular structures such as heparin, platelet and mitochondrial membranes, and DNA.^{17–21} In view of these data, several functions have been proposed for apo H through the link with charged phospholipids, e.g., inhibition of blood clotting and platelet prothrombinase activity.^{22,23} However, its physiological function is still unknown.

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The biochemical properties of apo H and our previous results on HBV binding led us to investigate the possibility that apo H binds to other viral proteins. The aim of this work was therefore to demonstrate the binding of apo H to HIV type 1 and type 2 proteins.

MATERIALS AND METHODS

Materials

Viral recombinant soluble proteins (rp26 of HIV-2 ROD and rp24, rp18, and rgp160 of HIV-1 LAI were kindly donated by Transgene S.A. [Strasbourg, France]). Bovine serum albumin (BSA) was purchased from Fluka (St. Quentin Fallavier, France) and 20% human albumin solutions were obtained from the Centre Régional de Transfusion Sanguine of Montpellier (Montpellier, France). Ultrapurified albumin, herein noted as uHSA, was obtained as described.²⁴ Ninety-six-well microtiter plates (Maxisorp) were from Nunc (Roskilde, Denmark). 9G1 and 8C3 monoclonal antibody to apo H was kindly donated by J. Arvieux.²⁵ Polyclonal antiserum to rgp160 was obtained as described.²⁶ ATB (antigen transfer buffer) and Sat1 and Sat3 saturation blot buffers were purchased from Labo-Centre S.E.L.A.R.L. (Béziers, France).

Isolation and purification of apolipoprotein H from human serum albumin

Apo H was purified from human plasma albumin solution as described.⁹ Briefly, a human albumin solution from Cohn plasma supernatant IV was applied to a column of dextran sulfated beads (Sigma, St. Louis, MO) prebalanced in 0.15 M NaCl. After rinsing with 10 mM phosphate buffer (pH 7.4)–0.2 M NaCl, a fraction was eluted with a 2 M NaCl step, then diluted 10-fold in a 10 mM sodium phosphate buffer, (pH 6.8) and loaded over a hydroxylapatite gel (IBF, Villeneuve-la-Garenne, France) prebalanced with the same buffer. The gel was then rinsed with the same buffer and the apo H eluted by increasing the ionic strength with 1 M KCl. The solution obtained was dialyzed against distilled water and lyophilized. At this stage, the purity of apo H provided a single band at 50 kDa, as checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 10%).²⁷

Coupling of apolipoprotein H and anti-apolipoprotein H antibody (c9G1) to alkaline phosphatase

Apo H was coupled to alkaline phosphatase as described.²⁸ Briefly, 500 µg of apo H was added to 4500 units of alkaline phosphatase (3000 U/mg; Boehringer GmbH, Mannheim, Germany) in the presence of 0.2% glutaraldehyde, followed by lysine and ethanolamine inactivation and exclusion chromatography over a Sephadex G25 gel column (Pharmacia, Orsay, France). Given concentrations of conjugated apo H related to the apo H alone.

9G1, a monoclonal antibody to apo H, was conjugated according to the same protocol as above.

Antiserum production against apolipoprotein H

One rabbit was immunized with 250 µg of purified apo H in Freund's complete adjuvant (Difco, Detroit, MI) and boosted

with the same amount 1 month later in Freund's incomplete adjuvant. Ten days later the rabbit was bled and the serum decanted, aliquoted, and cryopreserved.

Enzyme immunoassay using apolipoprotein H for the capture of viral antigen by apolipoprotein H

For HIV-1 antigen detection in HIV-1-positive patients and in healthy blood donor sera, 96-well microtiter plates were coated for 2 hr at 37°C with 1 µg of apo H per well in 100 µl of either 50 mM acetate buffer (pH 5.6) or phosphate-buffered saline (PBS), pH 7.1. After saturation with 200 µl of 2% BSA in PBS (pH 7.4) for 1 hr at 37°C and washing with PBS, 100 µl of serum diluted in either 50 mM acetate buffer (pH 5.6) or PBS (pH 7.1) was added. After a 90-min incubation at 37°C and four washings, 100 µl of biotinylated polyclonal anti-HIV-1 antibodies supplied in the ELAVIA (R) Ag I kit (Pasteur Diagnostics, Marnes-la-Coquette, France) was incubated at 37°C for 90 min. After washing, detection with streptavidin-peroxidase conjugate was done according to the manufacturer procedure (Pasteur Diagnostics). Wells coated with BSA or α_1 T glycoprotein alone were used as negative controls.

Alternatively, detection of serum viral antigens fixed on coated apo H was performed using a double-step procedure that included a 1-hr incubation at 37°C of a rabbit polyclonal antiserum to the rgp160 of HIV-1, diluted to 1:20,000 in PBS, followed by a second incubation with alkaline phosphatase-conjugated goat anti-rabbit IgG antibodies, revealed according to the manufacturer procedure (Promega, Charbonnières, France).

Ligand-blotting assays

Proteins were directly dotted in ATB to nitrocellulose by aspiration through a Multiblot-S apparatus (Millipore, Bedford, MA) before two 15-min saturation steps in Sat1 and Sat3 as described.²⁹ For further purification, rp26 was solubilized in SDS sample buffer, without 2-mercaptoethanol or dithiothreitol, separated by 12.5% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), then blotted by diffusion in ATB. The nitrocellulose was saturated as mentioned above. Commercial nitrocellulose strips containing HIV-1 or HIV-2 proteins (New LAV Blot I; Pasteur Diagnostics) were resaturated in the same manner. Nitrocellulose strips were incubated with apo H or conjugated apo H in 1 ml of 50 mM acetate buffer (pH 5.6) containing 0.1% hydrolyzed gelatin (Sigma) and 0.5% Triton X-100 for 2 hr at room temperature, then washed once in 50 mM acetate buffer (pH 5.6)–0.05% Triton X-100, and twice in PBS containing 0.05% Triton X-100. Conjugated apo H binding to viral proteins was revealed with nitro blue tetrazolium (NBT; Sigma) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP; Sigma) according to manufacturer instructions, except that Tris-HCl buffer at pH 8.8 instead of pH 9.5 was used.

Enzyme-linked immunosorbent assay

Unless otherwise noted, the binding assay of apo H to recombinant viral proteins was performed as follows: 96-well microtiter plates were coated for 2 hr at 37°C using 200 ng of viral proteins per well in 100 µl of PBS (pH 7.4). After saturation with 200 µl of Sat1 for 20 min, Sat3 for 10 min at 25°C, and washing with 0.05% Triton X-100 in PBS, 100 µl of conjugated apo H dilutions in 50 mM sodium acetate buffer (pH 5.6)

containing 0.5% Triton X-100 and 0.02% BSA was added and incubated for 90 min at 37°C. The plates were then washed six times and incubated with 4-nitrophenyl phosphate (4-NPP; Sigma) according to the manufacturer procedure. Absorbance was measured at 405 nm with a spectrophotometer (Titertek Multiskan Plus; Flow Laboratories, McLean, VA). The means of four replicates were plotted.

For the study of the pH dependence of conjugated apo H binding to viral recombinant proteins, buffer containing either 50 mM acetate at pH 5.2 and 5.6, or 17 mM phosphate at pH 6, 6.4, 6.8, 7.2, and 7.6, in both cases with sodium counterion, or 50 mM Tris-HCl at pH 8, 8.4, and 8.8, was used.

RESULTS

Capture by apolipoprotein H of HIV-1 antigenic proteins from patient sera

Two different approaches were used to detect possible binding of apo H to HIV-related antigens.

In the experiment shown in Fig. 1, 10 HIV-1-seropositive sera were chosen: sera 1 to 6 were AIDS and antigen positive (HIV-1 Ag⁺), whereas sera 7 to 10 were CDC stage II of HIV infection and antigen negative (HIV-1 Ag⁻). For these 10 sera, HIV-1 antigens had been tested using commercial conjugated polyclonal antibodies specific to Env and Gag proteins of HIV-1 (Pasteur Diagnostics). Different dilutions in PBS of each serum were added to apo H-coated microtitration plates. Captured HIV-1 antigens were then detected in the same way as described above. Strong positive signals were obtained for the 100- and 1000-fold dilutions in 5 of 6 samples from HIV-positive sera of patients 1 to 6, whereas significant responses at 10-fold dilution were observed only for two of them, sera 3 and 5. Negative signals were obtained for all four HIV antigen-negative sera at all dilutions. Control wells coated with BSA alone or α_1 T glycoprotein (results not shown) showed nega-

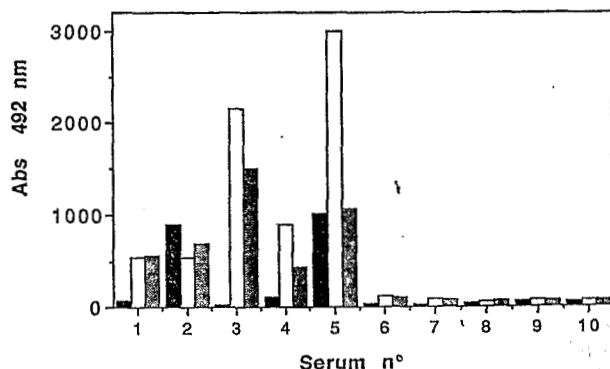


FIG. 1. Capture by coated apo H of HIV-1-related antigens in 10 HIV-1-seropositive patients sera: influence of serum dilution. Six HIV Ag⁺ sera (Nos. 1 to 6) and those of four HIV Ag⁻ (Nos. 7 to 10) were diluted 10-, 100-, and 1000-fold, respectively (represented by full, empty, and hatched bars, respectively) and tested in microtiter wells. HIV-1 antigens were detected in two steps, using commercial biotinylated polyclonal antibodies specific to Env and Gag proteins and streptavidin peroxidase.

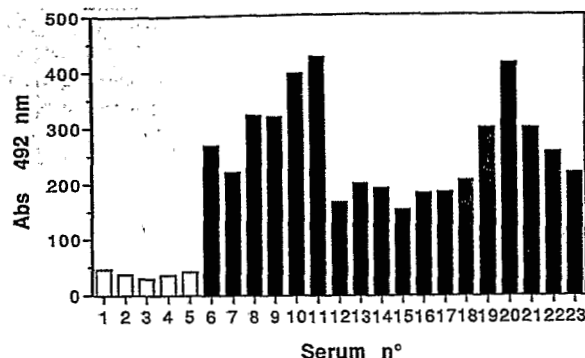


FIG. 2. Capture by apo H of HIV-1 Env-related antigens in AIDS patient sera. Sera (100-fold diluted) from five healthy donors (Nos. 1 to 5) and from 18 HIV Ag⁺ patients (Nos. 6 to 23) were tested. Antigens were detected by using a rabbit anti-gp160 polyclonal serum and alkaline phosphatase-conjugated goat anti-rabbit IgG.

tive responses for all sera, like those obtained from HIV-negative sera.

For the experiment shown in Fig. 2, a 100-fold dilution was chosen to screen sera from 18 HIV-1 antigen-positive AIDS patients and 5 healthy blood donors. But the detection was restricted to gp160-related antigens, by using an anti-gp160 rabbit polyclonal serum and conjugated anti-rabbit IgG antibodies in a two-step revelation procedure. It was seen that all of the HIV antigen-positive sera were positive for the presence of apo H-captured HIV envelope glycoprotein, while those from the five healthy donors were negative. We observed a better binding of viral antigen to apo H at pH 5.6 than at pH 7.1 (results not shown).

These results indicated that apo H was able to capture serum HIV-1-related antigens and incited an investigation into a possible direct binding between the apo H and diverse recombinant Env and Gag HIV proteins.

Effect of physicochemical parameters on the binding of apolipoprotein H to HIV proteins

In an ELISA system, we studied the influence of pH and ionic strength on the binding of apo H to recombinant viral proteins. The results in Fig. 3 show the pH dependence of the binding of conjugated apo H to rp18 of HIV-1 coated onto microtitration plates. In the first conditions without NaCl, significant binding of apo H to rp18 was observed within a pH range of 5.2 to 7.00, with an optimum around pH 5.6. On the addition of 0.15 M NaCl, the binding was notably decreased at all pH values. A similar reaction pattern was observed for rgp160 and rp26, with weaker levels, while no reaction or close to background was noted with either rp24 of HIV-1 or HSA, either at pH 5.6 or 7.0 (data not shown).

In another experiment (results not shown), the influence of NaCl concentration, from 0 to 1 M, during incubation of conjugated apo H on the coated recombinant protein was studied at pH 5.6 or 7.00: binding of conjugated apo H to rp18 slowly increased on addition of NaCl, reached a maximum at 0.05 M NaCl, and decreased with further NaCl.

Hence, pH 5.6 and 50 mM sodium acetate, being near the

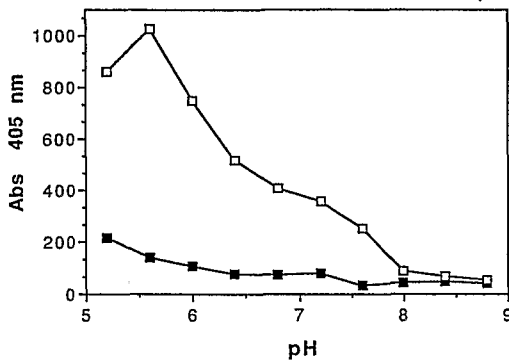


FIG. 3. Effect of pH on the binding of conjugated apo H to rp18 of HIV-1 LAI in the absence (open squares) or presence (solid squares) of 0.15 M NaCl.

optimum conditions, were chosen as standard conditions for further studies.

Specificity and dose response effect of binding

The specificity of these interactions was assessed in three ways, namely by studying the dose response, the homologous competition, and the inhibition by various specific antisera.

First, the capacity of apo H to interact with viral proteins in a dose-dependent manner was tested and controlled with non-HIV proteins such as HSA and BSA. As shown in Fig. 4, conjugated apo H bound, in a first-order-like reaction, to recombinant viral proteins p18, gp160 of HIV-1 LAI, and p26 of HIV-2 ROD. The reaction was the strongest for rp18 and decreased for rp26 and rgp160. A saturation plateau was reached for each retroviral protein at about 20 nM of the initial conjugated apo H. The binding was specific since the apo H did not bind to HSA or BSA or rp24 from HIV-1 LAI, even at 40 nM concentration of conjugated apo H.

Figure 5 shows the capacity of unconjugated pure apo H to inhibit binding of a constant amount of conjugated apo H. For each of the bound proteins, near 100% inhibition was reached with a 4 μ M concentration of initial apo H. Native unlabeled apo H exhibited an ID_{50} (50% inhibition dose) of 0.3 μ M for rgp160, 0.5 μ M for rp26, and 0.9 μ M for rp18.

Specificity was ascertained by using antibodies against apo

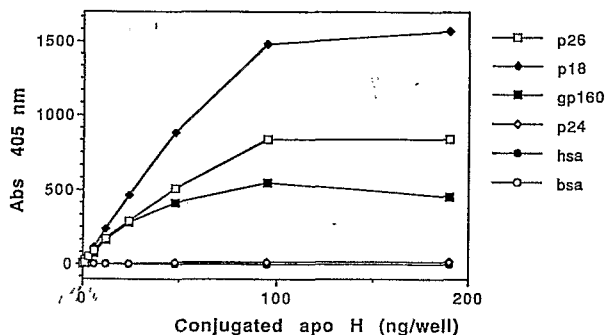


FIG. 4. Dose response and saturation of the binding of conjugated apo H to HIV proteins. Absorbance activity of conjugated apo H bound to rp18, rp26, rp24, rgp160, BSA, and HSA was plotted as a function of the initial conjugated apo H input.

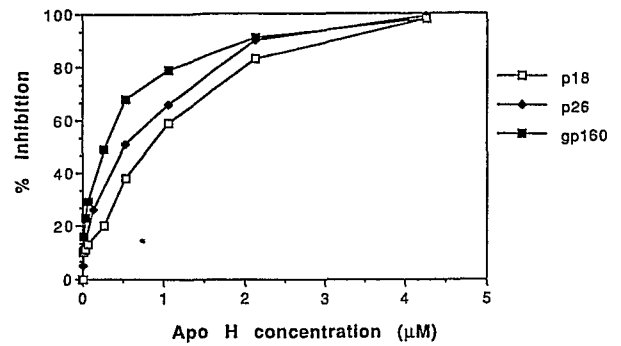


FIG. 5. Homologous competitive inhibition by unconjugated pure apo H. Eight nanograms of conjugated apo H was mixed with increasing concentrations of pure apo H, from 4 nM to 4.3 nM, in 100 μ l/well of reaction mixture. The results were plotted as percentages of the measures of conjugated apo H alone.

H and gp160. Figure 6 shows that a rabbit serum immunized against apo H and preincubated with it was able to decrease the binding to each of the three viral proteins, whereas the serum before immunization did not. Incubation of the viral proteins, coated onto microtiter wells, with an anti-gp160 serum abolished only the binding to the rgp160 and had no influence on the binding of apo H to the rp18 and rp26.

Ligand-blotting and -dotting assay experiments

Binding of conjugated apo H to HIV proteins was further tested with recombinant viral proteins adsorbed on nitrocellulose strips. About 200 ng of each viral protein (rp26 of HIV-2, rp24, rp18, and rgp160 from HIV-1) and human albumin as controls, either ultrapurified or Cohn's supernatant IV fraction, were dotted onto strips of nitrocellulose as described in Materials and Methods and incubated for 1 to 2 hr at 25°C in

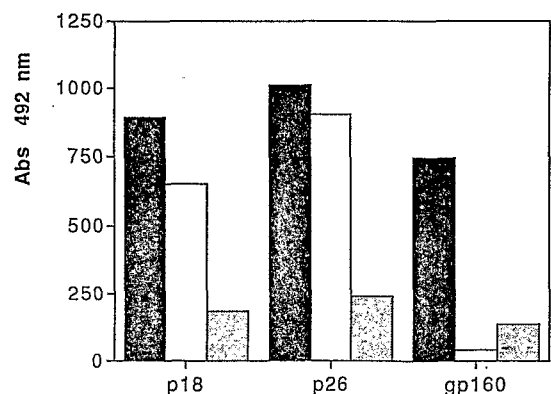


FIG. 6. Effect of anti-rgp160 and anti-apo H rabbit sera on the binding of conjugated apo H to diverse recombinant HIV proteins. Before being added to microtiter wells coated with recombinant viral proteins, 20 ng of conjugated apo H was preincubated for 1 hr at 22°C with a 1000-fold dilution of rabbit serum before and after immunization against apo H, showed by black and gray bars, respectively. In wells represented by white bars, a 1000-fold dilution of rabbit anti-rgp160 serum was incubated for 1 hr at 22°C with the coated viral proteins, following which 20 ng of conjugated apo H was added after four washes with PBS.

the presence of increasing concentrations of conjugated apo H. After the washes and detection, the alkaline phosphatase (AP) activity of the conjugated apo H, shown in Fig. 7, lanes 2 to 5, was strongly visible for rp18 and rp26, and weaker for rgp160, with a dose-response effect for each of them. No reaction was visible for the rp24 or the two different human albumin preparations used. In this experiment, apo H binding to viral proteins was detectable with a minimum conjugated apo H concentration of 4 ng/ml for rp18 and rp16 and 20 ng/ml for the rgp160. AP alone did not show any reactivity at concentrations up to 2.5 mg/ml (Fig. 7, lane 6).

A similar reactivity pattern was obtained by incubating unlabeled apo H, further recognized by the conjugated anti-apo H monoclonal antibody c9G1 in Fig. 7, lane 8. The background was likely due to the high initial apo H concentration of 1 mg/ml. Omission of apo H gave no reactivity at all (Fig. 7, lane 7). The rp26 preparation used throughout this study was claimed to be 80% pure, while the rp18 was claimed by the manufacturer to be more than 95% pure. To determine the binding specificity of apo H, this rp26 preparation was submitted to SDS-PAGE separation and the major component was identified as rp26 by using a specific antibody (not shown). After reaction with the conjugated apo H, the phosphatase activity again revealed the recombinant p26 (Fig. 7, lane 10). AP alone did not react (Fig. 7, lane 9).

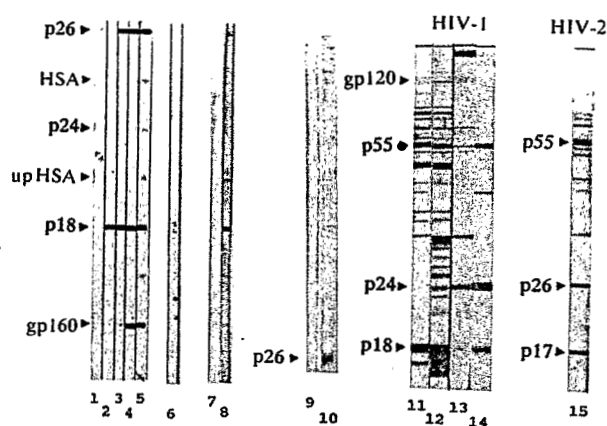


FIG. 7. Reaction of apo H with retroviral dotted and blotted proteins. In lanes 1 to 8, proteins were dotted as indicated. Lanes 1 to 5: conjugated apo H was reacted at concentrations of 0, 4, 20, 100, and 500 ng/ml, respectively. Lane 6: control reaction with AP alone (2500 ng/ml). Lanes 7 and 8: after a first incubation with, respectively, no apo H and 1 μ g of apo H per milliliter in 3 mM phosphate buffer (pH 6.8)–50 mM NaCl–0.5% Triton X-100, two washes with 50 mM Tris-HCl (pH 8.2)–50 mM NaCl, one with PBS, strips were incubated with 9G1 monoclonal antibody to apo H (1 μ g/ml) in PBS–0.1% Tween 20, then rinsed four times in the same buffer before detection. Lanes 9 and 10: Western blot strips containing electrophoresed recombinant p26 were incubated with, respectively, AP alone (1 mg/ml) and conjugated apo H (50 ng/ml). Lanes 11 and 12: two strips from different commercial Western blot lots of HIV-1 were reacted with conjugated apo H (50 ng/ml). Lanes 13 and 14: immunoblot control reactions with, respectively, a human HIV-positive control serum and serum from a rabbit immunized against a recombinant Gag protein containing peptides from p18 and p24. Lane 15: a commercial strip for HIV-2 Western blot was reacted with conjugated apo H (50 ng/ml).

By using commercial Western blot strips of HIV-1 and HIV-2, containing viral proteins pretreated under dissociating and reducing conditions, we confirmed the binding of conjugated apo H to proteins of HIV from cell culture supernatants, as shown in Fig. 7. There was substantial recognition of p18 and p55 of HIV-1, as well as of p17, p26, and p55 of HIV-2 (Fig. 7, lanes 11, 12, and 15). A number of possible precursors and catabolites reacted differently along the commercial lot strips. gp120 was scarcely visible (Fig. 7, lane 12). The lack of gp160 binding is discussed below.

In addition, we observed in ligand-dot experiments (not shown) that (1) reactions were stronger when each of the proteins was dotted separately, suggesting a possible competition between them toward apo H; (2) BSA and α_1 T glycoprotein used as controls did not react; (3) the specificity of conjugated apo H binding to rp26 was ascertained by inhibition with a rabbit anti-apo H serum, while the control nonimmune serum had no effect; (4) weaker but still visible binding of conjugated apo H to the three viral proteins occurred when acetate buffer was replaced by PBS (pH 6.8 to 7.00); and (5) detection of bound apo H using another monoclonal anti-apo H antibody, 8C3, in a three-step procedure, and labeled anti-mouse antibody, led to the same reactivity as the conjugated apo H.

We concluded that apo H interacted with some of the structural HIV proteins adsorbed onto the nitrocellulose: the p18 and p26 Gag proteins and the envelope gp160 protein, respectively, in this decreasing order, in the presence of the detergent Triton X-100, according to the results of ELISA experiments.

DISCUSSION

Among studies on virus-plasma interactions, this work demonstrated the capacity of apo H to bind preferentially, under given conditions, to retroviral HIV antigens, notably the p18 Gag protein of HIV-1, the p26 Gag protein of HIV-2, and to a lesser extent to gp160 of HIV-1. This binding was dependent on both pH and ionic strength, with an optimum at pH 5.6 and 50 mM NaCl, and is not sensitive to reduction of the viral proteins. The existence of a saturation dose and the inhibition either by homologous internal competition or by specific antisera demonstrated the specificity of the binding. Binding of apo H was also observed to the p55 precursor Gags of HIV-1 and HIV-2. In addition, this binding is not restricted to either recombinant or cell line-harvested viral proteins since apo H was also able to capture components from sera of HIV-infected antigen-positive patients. Our Western blot results showed a weak reaction of apo H to gp120 and a lack of reaction to the gp160, even though strong reactivity of apo H to this recombinant protein was observed in ELISAs and dot assays. This difference could be explained by taking into account the results reported by Pinter and by Zolla-Pazner, showing that gp160 and gp120 on commercial strips were mostly polymers of gp41.^{30,31} Preliminary experiments confirmed the binding of apo H to recombinant gp120.

The situation was not unique concerning the binding of apo H to HIV viral proteins. Other plasma proteins, including apo A-1, have been shown to bind HIV.⁵ In the case described here of

apo H binding to HIV proteins, the effects of NaCl concentration and pH involve electrostatic and hydrophobic interactions, and in this respect our results agree with those on the binding of apo H to phospholipids.¹⁷ The observed decrease in binding versus the increase in molecular weight could simply reflect a lesser number of molecules coated on the support. Values of around 0.5 μ M obtained for 50% inhibition in the competitive assay between native apo H and conjugated apo H for HIV recombinant antigens were close to those obtained for cardiolipin, thus indicating a rather high affinity for apo H for the three viral proteins.³² The binding of apo H to different viral antigens raises the question of its mechanism, especially in view of its polyspecificity toward antigens with no apparent primary sequence identity, although the same property has been reported for fibronectin, another plasma protein.³³ This polyspecificity, with respect to conformational changes and hydrophobic interactions, is discussed below.

Apolipoproteins are generally known for their conformational changes and interactions with lipids.^{34,35} It is known that highly flexible proteins such as apolipoproteins A-1, A-2, and C-1 absorb rapidly and reversibly at air-water interfaces, reflecting a change in their structures, while classic globular proteins interact more weakly and irreversibly with the interface. The above-mentioned interaction of apo A-1 with the N terminal of gp32 of SIV and gp41 of HIV, as well as with lipids, has been documented as being mediated by amphipathic α -helix regions.⁶ Human apo H was identified as a component of circulating plasma lipoproteins, especially chylomicrons and HDL forms.¹⁴ In contrast to other apolipoproteins, apo H does not associate itself in aqueous solution and its spectrum by circular dichroism did not show the existence of α helices, although analysis of the primary sequence using the Chou-Fasman algorithm indicated three regions with a putative α -helix structure in apo H.³⁶⁻³⁹ Moreover, the surface activity of the apo H, at neutral pH, had the same characteristics as typical globular proteins, while at acidic pH the absorption ratios at the air-water interface were similar to those found for apo A-1. These data indicate a modification of the secondary and tertiary structures.³⁶ Conformational changes for apo H after binding either to charged phospholipids or to solid surfaces have also been suggested.³⁹ For these reasons, we postulate that similar changes may have occurred under our conditions, especially at acidic pH between 5.2 and 6.8. Consequently, region(s) of apo H responsible for binding to viral proteins may have been unmasked.

Apo H has a strong affinity for negatively charged lipids, and a phospholipid-binding site has been identified and located in the fifth domain of apo H.^{17,32,40} Two binding sites on the mitochondria during the agglutination stage by apo H have been shown. One of these was inhibitable by charged phospholipids.⁴¹ The presence of lipids was described for the binding of apo H to HBsAg.⁴² We assume that apo H at least partially interacted through the effects of either detergents or lipids, which also induced conformational changes justifying the observed polyspecificity toward HBsAg and different HIV proteins as shown by the present results.³² Our experiments with the ligand-blotting assay support this hypothesis, since we observed strong reactions either by incubating the viral proteins with apo H in the presence of a detergent such as Triton X-100 or by adding the detergent to these proteins in a preincubation step only (results not shown).

It is noteworthy that HIV-1 and HIV-2 viruses are known to select specific lipid domains containing charged phosphatidic acid (PA) and phosphatidylserine (PS) from the plasmatic membrane of the host cell during the budding process. Therefore, PS and PA levels are higher in the viral envelope than in the membrane of infected cells and the cholesterol/lipid ratio is also 2.5-fold higher than at the surface of the host cell.⁴³ Since interactions between HIV-1 Gag proteins and acid phospholipids and membrane lipids have been described, interactions between retroviral HIV proteins and apo H could result from apo H affinity for lipids.⁴⁴ These data fit the observed preferential binding of apo H to some viral proteins, promoted through either the addition of detergent or the presence of native lipids in proteins isolated from sera. Finally, the differences in phosphatidylinositol and phosphatidylserine levels observed in HIV-2 and HIV-1, resulting in charge and hydrophobicity differences, could induce different reactivities of p26 and p24 to apo H.⁴³

Moreover, when micelles are made by adding lipids to purified retroviral HIV-1 proteins in the presence of detergents, to form virosomes, gp160 and p18 of HIV-1 are included in these virosomes, while p24 is not.⁴⁵ This is consistent with the observed lack of reaction of p24, in contrast to p18 and gp160, to apo H. Finally, compounds such as detergents or lipids could mediate conformational changes of apo H or viral proteins considered as targets, or both apo H and target, and induce complex apo H/target formation, brought out in this work. The detergent would therefore allow the binding of conjugated apo H to the recombinant viral proteins under rather nonphysiological conditions of pH and ionic strength, whereas the native lipids present on the viral proteins of HIV-infected sera would bind to the purified apo H under more physiological conditions (PBS), conditions used in our work for the antigen capture in patient sera. This field is being investigated.

Under our conditions, the binding of apo H to serum viral components detected by the use of anti-gp160 antibodies and 100-fold diluted patients sera is consistent with a relatively high concentration of soluble envelope glycoprotein (up to 100 ng/ml) measured in sera from AIDS and ARC patients.⁴⁶ The decreasing response at high serum concentration reflects perhaps a homologous competition with the serum apo H or the presence of inhibitors, such as antibodies. Anti-phospholipid antibodies have been detected in HIV-infected patients.⁴⁷ Would some of these, in the presence of plasmatic apo H, involve nonspecific reactions with viral proteins (especially p18) to give some false-positive anti-HIV reactions?⁴⁸ Is apo H present in the serum able to interfere with some of the conventional diagnostic methods?

A precise functional role has not yet been assigned to apo H. However, involvement was suggested during HIV infection, by either mediation or modulation of the recognition of HIV-induced apoptotic lymphocytic debris by macrophages.⁴⁹ More generally, does apo H interfere *in vivo* during the infection? Does it inhibit syncytium formation and viral infectivity like apo A1? Current work is being undertaken in these directions.

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