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α_1-Acid glycoprotein binds human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein via *N*-linked glycans

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In the present study, we demonstrate a specific low-affinity interaction between recombinant precursor gp160 (rgp160) or surface unit gp120 (rgp120) of human immunodeficiency virus type 1 (HIV-1) and α_1-acid glycoprotein (AGP), a human glycoprotein displaying complex type *N*-glycans. Binding of rgp160/rgp120 to agarose-coupled AGP was dose-dependent, saturable, calcium-, pH- and temperature-dependent. Binding was inhibited by soluble AGP, asialo-AGP, fetuin, β-D-GlcNAc$_{47}$-BSA, α-D-Man$_{20}$-BSA, mannan, complex-type asialo-agalacto-tetraantenary precursor oligosaccharide from human AGP and oligomannose 9 from porcine thyroglobulin; fully deglycosylated AGP was not inhibitory. The three AGP glycoforms separated on immobilized ConA bound rgp160 to the same extent as did unfractionated AGP. These findings extend our previous results on the carbohydrate-binding properties of HIV-1 envelope (*Env*) glycoprotein in that they demonstrate the involvement of AGP glycan moieties in the binding to rgp160/rgp120. Preincubation of rgp160 with AGP or mannan significantly reduced its binding to monocyte-derived macrophages (MDM), suggesting that AGP may play a role in preventing binding of soluble or virus-bound *Env* glycoprotein to CD4$^+$ monocytic cells.

Keywords: *Env* glycoproteins, HIV-1, α_1-acid glycoprotein, *N*-linked glycans

Introduction

The envelope glycoprotein (*Env*) of human immunodeficiency virus type 1 (HIV-1) is synthesized as a 160 kDa precursor (gp160), which is subsequently cleaved into outer-membrane gp120 and transmembrane gp41 [1, 2]; gp160 is heavily glycosylated, and *N*-linked glycans represent about 50% of its molar mass [3–5]. The role of glycans in the pathophysiology of HIV-1 infection is currently under investigation [6–8]. We have recently shown that gp160 and processed gp120 display specific carbohydrate-binding properties [9, 10]: they recognize *N*-acetyl-glucosamine residues of oligosaccharidic structures, oligomannose glycans and the mannosyl core of complex type *N*-linked glycans such as, for example, those presented by mannan, thyroglobulin, fetuin or asialofetuin.

We hypothesized that these carbohydrate-binding properties may be involved in biological interactions with a human serum glycoprotein such as human α_1-acid glycoprotein (AGP or orosomucoid). AGP is characterized by a high carbohydrate content (42%) [11], with five *N*-linked bi-, tri- or tetra-antennary glycans per molecule [12, 13]. It can be separated into nonreactive, weakly reactive and reactive fractions by concanavalin A (Con A)-Sepharose chromatography [14]. The ability of Con A, a mannose-specific lectin, to bind glycopeptides presenting two antennae linked to the core pentasaccharide has been described [15]. Ogata *et al.* [16] found that the presence of at least two α-mannosyl residues with free hydroxyl groups at C-3, 4 and 6 is required for oligosaccharides to be retained by Con A. AGP is an acute-phase reactant, and as such its plasma level increases two- to four-fold in response to infection and inflammation [17]; an increase in the proportion of Con A-unreactive fraction is observed during pregnancy and liver damage, and Con A- reactive variants increase during acute inflammatory disorders [18]. Although the physical and chemical properties of AGP have been extensively described, its biological role remains poorly understood. It binds to various substances [19] and it appears to have a modulatory activity on the immune response [20]. Con A-reactive AGP has been reported to bind to the Man/GlcNAc specific macrophage lectin [21]; this might imply that each AGP variant has a different immunoregulatory property.

The aim of this study was to investigate whether AGP could bind to gp160/120 and possibly interfere with the binding of *Env* glycoprotein to CD4$^+$ cells.

Materials and Methods

Recombinant gp160 and gp120 Soluble recombinant gp160 (rgp160) of HIV-1(LAI) [22] purified to 90% homogeneity (a gift from PASTEUR MERIEUX Sérums et Vaccins, Lyon, France) was produced by BHK-21 cells infected with recombinant gp160 vaccinia virus as described [1]. This rgp160 has the same characteristics as rgp120 regarding the binding to

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CD4 [6]. Because of this, in some experiments only rgp160 was used. Soluble rgp120, > 90% pure, was a gift from the MRC AIDS Directed Programme (South Mimms, UK).

Radiolabelling was performed by the iodogen method as described [6]. Iodinated glycoproteins were separated from $Na^{125}I$ by filtration through a Sephadex G-25 (PD10) Column (Pharmacia, Uppsala, Sweden). Specific activity was 1.1 MBq μg^{-1}.

Homogeneity of the preparation was assessed by SDS-PAGE (4–20%). It was also verified that the labelled glycoproteins were still immunoreactive: HIV-positive human antisera (2 μl diluted 1:10 or 1:100) were dotted on to nitrocellulose filters; after saturation with 2 ml of phosphate-buffered saline (PBS), 5% bovine serum albumin (BSA, Sigma Chemical Co, St Louis, MO), for 1 h at 37°C to prevent non-specific binding, and washing with PBS-0.5% BSA, 0.02% Tween 20 (Sigma), the strips were incubated for 1 h at 37°C with [^{125}I]rgp160 or [^{125}I]rgp120, washed twice with PBS- 0.5% BSA, Tween 0.02%, and then autoradiographed.

Binding of rgp160/rgp120 to α_1-acid glycoprotein-agarose (AGP-agarose) AGP coupling to cyanogen bromide-activated Sepharose-4B (Pharmacia), 6 mg of AGP per ml of gel, was carried out as recommended by the manufacturer. The binding of [^{125}I]rgp160 or [^{125}I]rgp120 to AGP-agarose was determined as follows: 10 μl of packed affinity matrix were suspended in 500 μl of buffer, 0.02 M Tris. 0.15 M NaCl, 0.01 M $CaCl_2$, 0.05% BSA, pH 7.4 (Tris-Ca-BSA), centrifuged and washed twice in 500 μl of buffer. Incubation of the matrix with various concentrations of radiolabelled glycoproteins ($0.1–5 \times 10^{-10}$ M) was for 1 h at 37°C, i.e. under equilibrium conditions since longer incubations did not increase the binding; the matrix was then washed twice in 500 μl buffer and solid phase-bound radioactivity was counted in a γ counter (LKB). As a negative control, rgp120/160 was incubated with non glycoprotein-derivatized CNBr-activated Sepharose. Results were expressed as mean values of duplicates.

The physicochemical characteristics of the interactions were analysed by performing the assays under different conditions: pH from 6.4 to 7.8 (experiments outside this range were not performed in order to avoid rgp160/rgp120 precipitation or denaturation); buffer without or with different $CaCl_2$ concentrations, or with 7.5 mM $MgCl_2$ or 7.5 mM EDTA (disodium Salt), both from Sigma; different temperatures (4, 20 and 37°C).

Carbohydrate specificity of rgp160/rgp120 binding to AGP-agarose To determine the carbohydrate specificity of the interaction, radiolabelled glycoproteins ($1–3 \times 10^{-10}$ M) were preincubated in Tris-Ca^{2+}-BSA for 45 min at 37°C with the following carbohydrates, carbohydrate derivatives, or glycoproteins (all from Sigma): D-galactose, *N*-acetyl-D-glucosamine, D-mannose, methyl α-D-galactopyranoside were tested at mM concentrations; D-mannan and β-D-glucan at 2 mg ml^{-1}; AGP and fetuin in the μM range. BSA (30 μM) was used as a control. Synthetic neoglycoproteins were tested up to 30 μM. The sugar/BSA substitution ratio was 17 for β-D-Gal-BSA and 47 for β-D-GlcNAc-BSA [9] (gifts from D. Bladier), 20 for α-D-Man-BSA and β-D-Lac-BSA (gifts from M. Monsigny), and 15 for melibiosyl-BSA (Sigma). Chitotriose (E.Y. Labs Inc., USA.) was also used up to 30 μM. In some experiments, 0–30 μM concentrations of the following compounds (Oxford Glycosystems, UK) were used: complex-type asialo-agalacto-tetraantennary oligosaccharide prepared by exo-glycosidase digestion of human AGP; oligomannose 9 from porcine thyroglobulin; fucose-substituted complex-type asialo-agalacto biantennary core from porcine thyroglobulin and complex-type asialo-galactosylated triantennary oligosaccharide from bovine fetuin.

AGP and mannan (Sigma) (both at 20 mg ml^{-1}) were used in experiments devised to assess reversion of rgp160 or rgp120 binding to the matrix: 100 μl of [^{125}I]rgp160/rgp120 (2 and 7×10^{-10} M, respectively) were incubated for 1 h at 37°C with 200 μl of the matrix in Tris-Ca^{2+}-BSA. Unbound rgp160/rgp120 was washed out with buffer until no significant radioactivity was detected; matrix-bound rgp160/rgp120 was incubated with 500 μl of AGP or mannan diluted in the same buffer at 37°C for 2–20 h. The supernatants were characterized by SDS-PAGE (4–20%)

Deglycosylation of AGP Two glycosidases were used: sialidase (EC 3.2.1.18) (Behringwerke, Marburg, Germany) and endo F-*N*-glycanase F (Boehringer Mannheim, Mannheim, Germany). AGP (10 mg ml^{-1} in PBS pH 7.4) was desialylated by adding 0.3 U of sialidase (300 μl in 0.05 M $NaHCO_3$, 0.15 M NaCl, 0.009 M $CaCl_2$, pH 5.5) for 2 h at 37°C. Free sialic acid (36 μg per mg AGP) was quantified by the thiobarbituric acid reaction [23]. AGP was then applied to a PD10 column (Pharmacia Fine Chemicals) in PBS pH 7.4 in order to remove free sialic acid.

Oligosaccharides were removed under nondenaturing conditions by addition of 3 U of endoglycosidase F/N glycosidase F to 5 mg of AGP in PBS (500 μl). The samples were incubated for 24 h at 37°C, and then submitted to gel filtration on a PD10 column in PBS.

Intact, desialylated or endoglycosidase-treated AGP preparations were analysed by SDS-PAGE (4–20%) under reducing conditions in a Tris buffer system according to Laemmli [24]. The effect of glycosidase treatment of AGP on [^{125}I]rgp160 interaction with AGP-agarose was evaluated. As a control, it was verified that the boiled enzymes incubated without AGP-derived products under the same conditions had no effect on rgp160 interaction with the matrix.

In other experiments, aliquots (2 μg of each) of intact, desialylated or endoglycosidase-treated AGP were dotted on to nitrocellulose strips. After 30 min at 20°C, the strips were saturated for 1 h at 37°C with Tris-Ca^{2+}, 5% BSA, pH 7.4, to prevent non-specific protein binding. Excess BSA was washed out with Tris-Ca^{2+}, 0.5% BSA, 0.02% Tween 20, pH 7.4, and strips were incubated with [^{125}I]rgp160 (1×10^{-10} M) for 1 h at

37°C. As controls, the binding of [^{125}I]rgp to spots of Con A (2 μg) or PNA (*Arachis hypogaea* agglutinin, 2 μg), both from Sigma, on nitrocellulose was also examined. In some experiments, [^{125}I]rgp160 was preincubated for 1 h at 37°C with mannan, β-D-glucan or AGP (20 mg ml^{-1}). [^{125}I]rgp160 binding to the nitrocellulose was revealed by autoradiography. Autoradiograms were obtained by exposing the nitrocellulose strips to Kodak GBX-2 films (New York) for 24 h at –80°C.

In other experiments, 2 μg of intact, desialylated or endoglycosidase-treated AGP were dotted on to nitrocellulose strips as described above and then incubated for 1 h at 20°C with peroxidase-labelled 50 nM Con A (Sigma). Diaminobenzidine (Sigma) in PBS, 1% H_2O_2 was used for staining.

Fractionation of AGP variants by affinity chromatography on immobilized Con A Fractionation was performed according to (25) AGP (20 mg in one ml of Tris-Ca^{2+}) was chromatographied on a Con A-Sepharose 4B column (Pharmacia) (30 × 0.8 cm) at 20°C; after incubation for 1 h at 37°C, the column was washed with Tris-Ca^{2+}-BSA at a flow rate of 0.1 ml min^{-1}. Unbound AGP (Con A-unreactive AGP A, about 52% of applied AGP) was eluted after one column volume of the buffer. Retarded AGP (AGP B, about 36%) was eluted slowly with a total volume of about 10 column volumes of the buffer. Bound AGP (Con A-reactive AGP C, about 12%) was eluted with 0.15 M methyl α-Man in the buffer. The collected fractions were desalted on PD10 in water and freeze-dried. The three fractions were analysed by SDS-PAGE. Their effects on rgp160 interaction with AGP-agarose were determined.

Cells Monocytes were prepared from blood or buffy coat of healthy volunteers of the Avicenne or the Pitie-Salpetriere Blood Banks by Ficoll-Hypaque (Pharmacia) separation, and were purified by adherence as previously described [29]. Peripheral blood mononuclear cells (PBMC), adjusted to 5 × 10^6 cells per ml, were layered on to 16 mm flat-bottomed wells (Costar, Cambridge, MA) and cultured for 5 days according to Valentin *et al.* [26]. Cells were then washed three times with Ca^{2+}-, Mg^{2+}-free PBS to remove non-adherent cells, and adherent cells were incubated for 24–48 h in fresh complete medium. Cell viability (Trypan blue exclusion) was then >95%. Adherent monocyte-derived macrophages (MDM) remained viable for more than 1 month. More than 90% of the adherent cells were shown to be monocytes by direct or indirect immunofluorescence staining with various monoclonal antibodies (mAbs) or isotype-matched controls.

CEM cells, a lymphoid human cell line, are $CD4^+$ and are susceptible to HIV infectivity [27]. They were maintained in culture under conditions described for PBMC with 10% FCS but without human AB serum.

Binding of rgp160 to the cells Adherent MDM (5 × 10^5 cells) in 500 μl of Hank's medium supplemented with 0.05% BSA and 2.5 mM $CaCl_2$ (Hank's-Ca^{2+}) were incubated for 2 h at 4°C with 500 μl of [^{125}I]rgp160 (2–6 × 10^{-10} M). After two washes by centrifugation (700 × **g**, 10 min, 4°C), cell-bound radioactivity was measured in a γ counter (Packard).

The effect of AGP (0–50 × 10^{-6} M) or mannan (2 mg ml^{-1}) on rgp160 binding to the cells was studied as follows: AGP was preincubated with [^{125}I]rgp160 in Hank's-Ca^{2+} for 1 h at 37°C prior to incubation with the cells for 3 h at +4°C; or AGP was preincubated with the cells for 1 h at 37°C prior to incubation with [^{125}I]rgp160 for 3 h at +4°C. Controls (2 mg ml^{-1}) with dextran, β-D-glucan and BSA were performed in parallel.

Binding of [^{125}I]rgp160 to the CEM cells was analysed by the same procedure but in 200 μl of buffer.

Statistical analysis Differences between mean values were evaluated by the Student *t* test.

Results

Binding of rgp160/rgp120 to AGP-agarose We have previously shown that HIV-1 viral gp120 and recombinant gp160 have specific β-D-*N*-acetyl-glucosaminyl/ mannosyl binding properties [9, 10]. To extend these findings, we examined [^{125}I]rgp160/rgp120 interaction with AGP-agarose (AGP is a natural human glycoprotein). Both rgp160 and rgp120 bound to AGP-agarose in a dose-dependent and saturable manner under equilibrium conditions (Fig. 1a, b). Under the same conditions, rgp120/160 bound poorly to non-glycoprotein-derivatized CNBr-activated Sepharose (Fig. 1a). In the light of these results, we chose the 50% saturation values of rgp160 and rgp120, 2.6 × 10^{-10} M and 1.2 × 10^{-10} M respectively, for further experiments. The characteristics of these interactions were analysed. In both cases, binding to the matrix was calcium-dependent, highly significant at 6 mM Ca^{2+}, and plateaued at 7.5 mM (Fig. 1c); binding was not influenced by the addition of $MgCl_2$ in a 7.5 mM calcium supplemented buffer and it was abolished in the presence of 7.5 mM EDTA added before initiation of the reaction (Fig. 1d). This indicates the specific calcium dependence of binding. Moreover, the binding was highly dependent on: (i) the temperature, low at both 4 and 20°C and strongest at 37°C (Fig. 1e); (ii) the pH, with binding increasing from pH 6.4 to 7.8 for rgp120 and from pH 6.4 to 7 and plateauing at pH 7.8 for rgp160 (Fig. 1f).

Carbohydrate specificity of rgp160/rgp120 binding to AGP-agarose In Table 1 are shown the mean 50% inhibiting concentrations (C_{50}) and maximum percentage inhibitions noted when different soluble carbohydrate structures were preincubated with [^{125}I]rgp160 or [^{125}I]rgp120 before addition to AGP-agarose. Rgp160 or rgp120 binding was inhibited in a dose-dependent manner by mannan (Fig. 2a), β-D-$GlcNAc_{47}$-BSA (Fig. 2b) and β-D-Man_{20}-BSA (Fig. 2e). β-D-Gal_{17}-BSA had an insignificant, highly variable effect as did β-D-Lac_{20}-BSA, while melibiosyl$_{15}$-BSA had no effect at all (Table 1, Fig. 2e). The monosaccharides and their glycosides were not inhibitory, even at concentrations up to 60 mM. In addition,

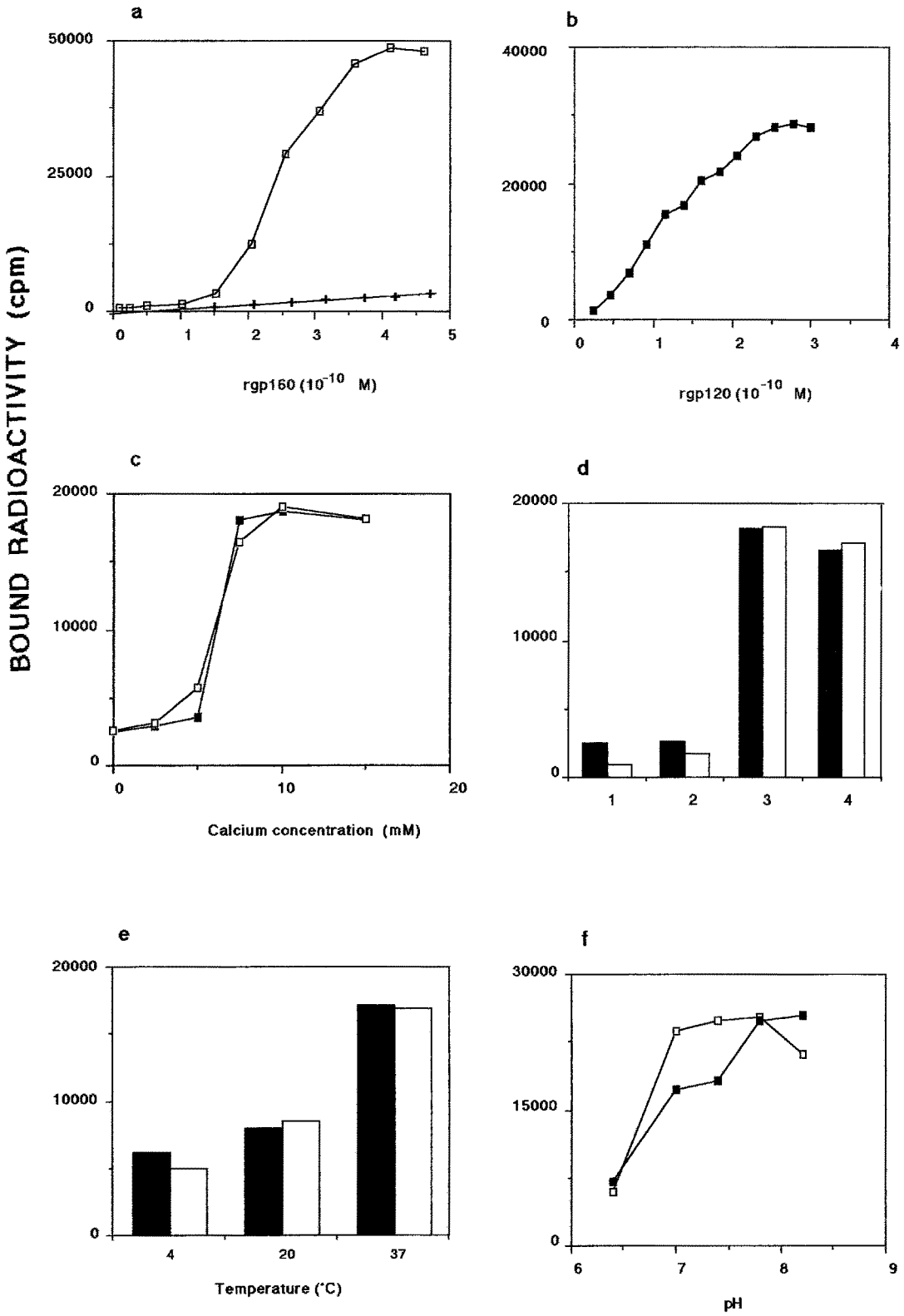

Figure 1. Influence of the physico-chemical conditions on rgp 160 (□) and rgp120 (■) binding to AGP-agarose. (**a**,**b**) rgp160 or rgp120 concentration: 0.1 to 5 × 10^{-10} M of [^{125}I]rgp 160 or [^{125}I]rgp120 were incubated for 1 h at 37°C with 10 μl of matrix in Tris-Ca-BSA (pH 7.4); in (**a**) [^{125}I]rgp160 was incubated under the same conditions with non glycoprotein-derivatized CNBr-activated Sepharose (+). (**c**) effect of the Ca^{2+} concentration on rgp120/160 binding; (**d**) effect of Ca^{2+} on the binding: lane (1) $CaCl_2$-free Tris-BSA; lane (2) in 7.5 mM $CaCl_2$ supplemented either with 7.5 mM EDTA or lane (3) with 7.5 mM $MgCl_2$; lane (4) in 7.5 mM $CaCl_2$. (**e**) Effect of the temperature; (**f**) effect of the pH. Representative results of at least three independent experiments.

Table 1. Inhibition of [^{125}I]rgp160 or [^{125}I]rgp120 binding to AGP-agarose by different carbohydrate compounds.

	C_{50}		Maximum % inhibition	
Compound	*rgp160*	*rgp120*	*rgp160*	*rgp120*
Mannan	0.5 mg ml^{-1}	0.5 mg ml^{-1}	84 ± 8	84.5 ± 7
β-D-glucan			0	0
Dextran			0	0
Chitotriose			0*	
	Simple carbohydrates			
Man			0	0
D-Gal			0	0
GlcNAc			0	0
Me-α-Clc			0	0
Me-α-Gal			0	0
	Neoglycoproteins			
β-D-Gal-BSA			29 ± 26	31 ± 22
β-D-Lac-BSA			39 ± 3*	
β-D-GlcNAc-Bsa	5 μM	6 μM	86 ± 5	90 ± 3
α-D-Man-BSA	6 μM		87 ± 3*	
Melibiosyl-BSA			0	0
	Natural glycoproteins			
AGP	25 μM	28 μM	92 ± 3.5	84 ± 4
Fetuin			83 ± 10	
BSA			0	0

Results are expressed as mean % inhibition ± SE of four to six independent experiments (except *n = 2). Concentrations for maximum % inhibition were 2 mg ml^{-1} for mannan, 30 μM for β-D-GlcNAc$_{47}$-BSA and α-D-Man$_{20}$-BSA, 100 μM for AGP, 20 μM for fetuin. Concentrations of dextran, chitotriose and β-D-glucan were 2 mg ml^{-1}; Man, D-Gal, Me-α-Glc, Me-α-Gal were tested at 60 mM concentration, β-D-Gal$_{17}$-BSA, melibiosyl-BSA and BSA at 30 μM concentration.

glycoproteins, such as AGP (Fig. 2c) and fetuin (Table 1), were also strong inhibitors. BSA, β-D-glucan, dextran or chitotriose had no effect. We did not estimate the intrinsic dissociation constant of rgp160 or rgp120/carbohydrate complexes [27] but, considering the C_{50} values reported in Table 1, rgp160 and rgp120 interact specifically with AGP with low affinity. Complex-type asialo-agalacto tetraantennary precursor oligosaccharide of AGP and oligomannose 9 from porcine thyroglobulin at 30 μM inhibited rgp160 binding by 70% and 63% respectively (n = 2) (Fig. 2f, Fig. 3a, b); complex-type fucose-substituted asialo-agalacto biantennary core from porcine thyroglobulin and complex-type asialo-galactosylated triantennary oligosaccharide from bovine fetuin inhibited binding by 40% and 30% respectively (n = 2) (Fig. 2f).

AGP reversed 80% of rgp160 or rgp120 binding (Fig. 4a,b) as did mannan (data not shown), but not BSA nor the buffer. This resulted in 80–85% of rgp160 and rgp120 being specifically eluted with either 20 mg ml^{-1} AGP or mannan. The eluted molecules exhibited the same migration patterns on SDS-PAGE as original rgp160 or rgp120 with estimated MW of 155 and 117 kDa, respectively (Fig. 4c). These results confirm the occurrence of specific interactions between rgp160/rgp120 and AGP.

Altogether, these data indicate that rgp160 and rgp120 specifically interact with both mannosyl and β-D-*N*-acetyl-glucosaminyl residues present on AGP. To further study the influence of the carbohydrate moieties on the interaction with rgp160, AGP was treated with sialidase or endo F/N glycanase. Such treatment decreased the apparent MW of AGP to 39 and 27 kDa, respectively (Fig. 5a). Endoglycosidase-treatment of AGP abolished its binding to peroxidase-labelled Con A (Fig. 5b). The results of Fig. 2d show that desialylated AGP also exhibited strong inhibitory effects on the binding of rgp160 to AGP-agarose, with a C_{50} value slightly lower than that observed with native AGP. In contrast, deglycosylation of AGP totally abolished its inhibitory effect (Fig. 2d). These results indicate that the presence of carbohydrate chains is a critical factor for the interaction of rgp160 with AGP. This involvement of carbohydrates in rgp160-AGP binding was also supported by the results of dot-spot analysis, using either intact, desialylated or deglycosylated AGP and [^{125}I]rgp160. Dot spot gave evidence that deglycosylated AGP did not interact with rgp160 while confirming that intact and desialylated AGP bound rgp160 (Fig. 5c). Con A (known to interact with rgp160) and PNA (which does not interact with rgp160) were used as positive and negative controls, respectively (Fig. 5c). Preincubation of [^{125}I]rgp160 with mannan (Fig. 5d) or AGP (Fig. 5e) abolished its binding to AGP dotted on to nitrocellulose. β-D-glucan had no effect (Fig. 5f).

Comparative effect of AGP variants on rgp160 binding to AGP-agarose The inhibitory effects of all three AGP variants separated on Con A-Sepharose did not significantly differ (n = 3; data not shown). These results indicate that, if AGP binding to rgp160 is essentially related to the presence of glycans on AGP, it is not influenced by the different proportions of bi-, tri- or tetra-antennary chains bound to the polypeptide backbone.

Inhibition of rgp160 binding to cells Adherent mononuclear cells were characterized by immunolabelling with different mAbs. As reported [29], 92 ± 2% of the cells were CD14$^+$ macrophages that expressed low CD4 levels but neither CD3 nor CD19 T and B cell markers, respectively. Figure 6 shows that radiolabelled rgp160 binding to the MDM is influenced by rgp160 concentration. The low percentage of rgp160 bound to these cells is in agreement with that previously observed by us [29] and by others [43]. When the cells were preincubated with AGP or mannan, no inhibition of rgp160 binding to the cells was observed (data not shown). However, preincubation of rgp160 with AGP or with mannan, significantly diminished

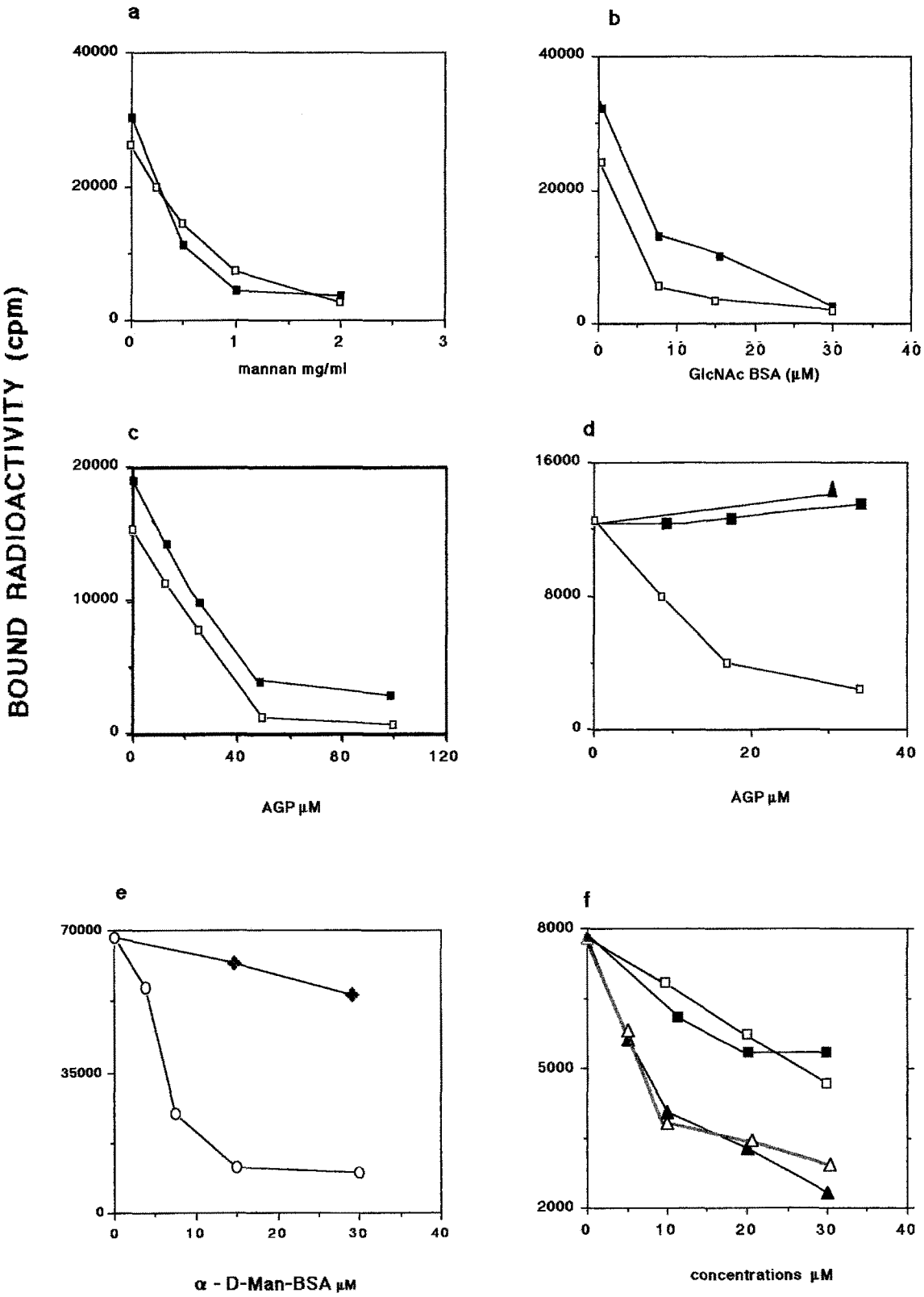

Figure 2. Inhibition of rgp160 (□) or gp120 (■) binding to AGP-agarose by: (**a**) mannan; (**b**) β-D-GlcNAc$_{47}$-BSA; (**c**) AGP; (**d**) inhibition of rgp160 binding by deglycosylated AGP (■), desialylated AGP (□), or BSA (▲); (**e**): α-D-Man$_{20}$-BSA (○) and β-D-Lac$_{20}$-BSA (❖) as control; (**f**): complex-type asialo-agalacto tetraantennary oligosaccharide of AGP (– ▲ –) and oligomannose 9 from porcine thyroglobulin (– △ –); complex-type fucose-substituted asialo-agalacto biantennary core from porcine thyroglobulin (– □ –) and complex-type asialo-galactosylated triantennary oligosaccharide from bovine fetuin (– ■ –).

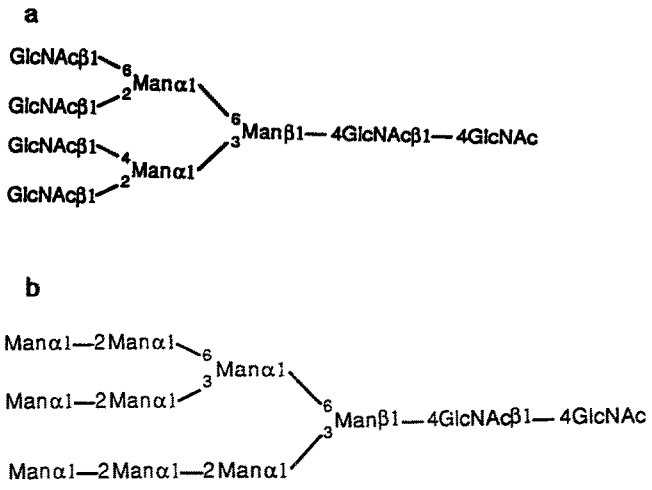

Figure 3. Structures of complex-type asialo-agalacto tetraantennary oligosaccharide from AGP (**a**) and oligomannose 9 from porcine thyroglobulin (**b**).

its binding to the MDM (Fig. 6 and Table 2). Dextran or β-D-glucan had no effect (Table 2). In contrast, at similar experimental conditions, AGP or mannan had no effect on binding of [^{125}I]rgp160 to CD4$^+$ cells of the CEM lineage (data not shown).

These findings suggest that AGP can inhibit the binding of HIV-1 *Env* glycoproteins to CD4$^+$ monocytic cells through its interaction with gp120/160, which apparently is not the case with respect to transformed lymphoïd CEM cells.

Discussion

In this study, we show that rgp160 and rgp120 binds to AGP. We demonstrate that the binding of rgp160 and rgp120 to an AGP-agarose affinity matrix is specific, saturable, pH-, temperature- and calcium-dependent, characteristics that are common to lectin-ligand interactions [30]. We extend our previous results on the carbohydrate binding properties of

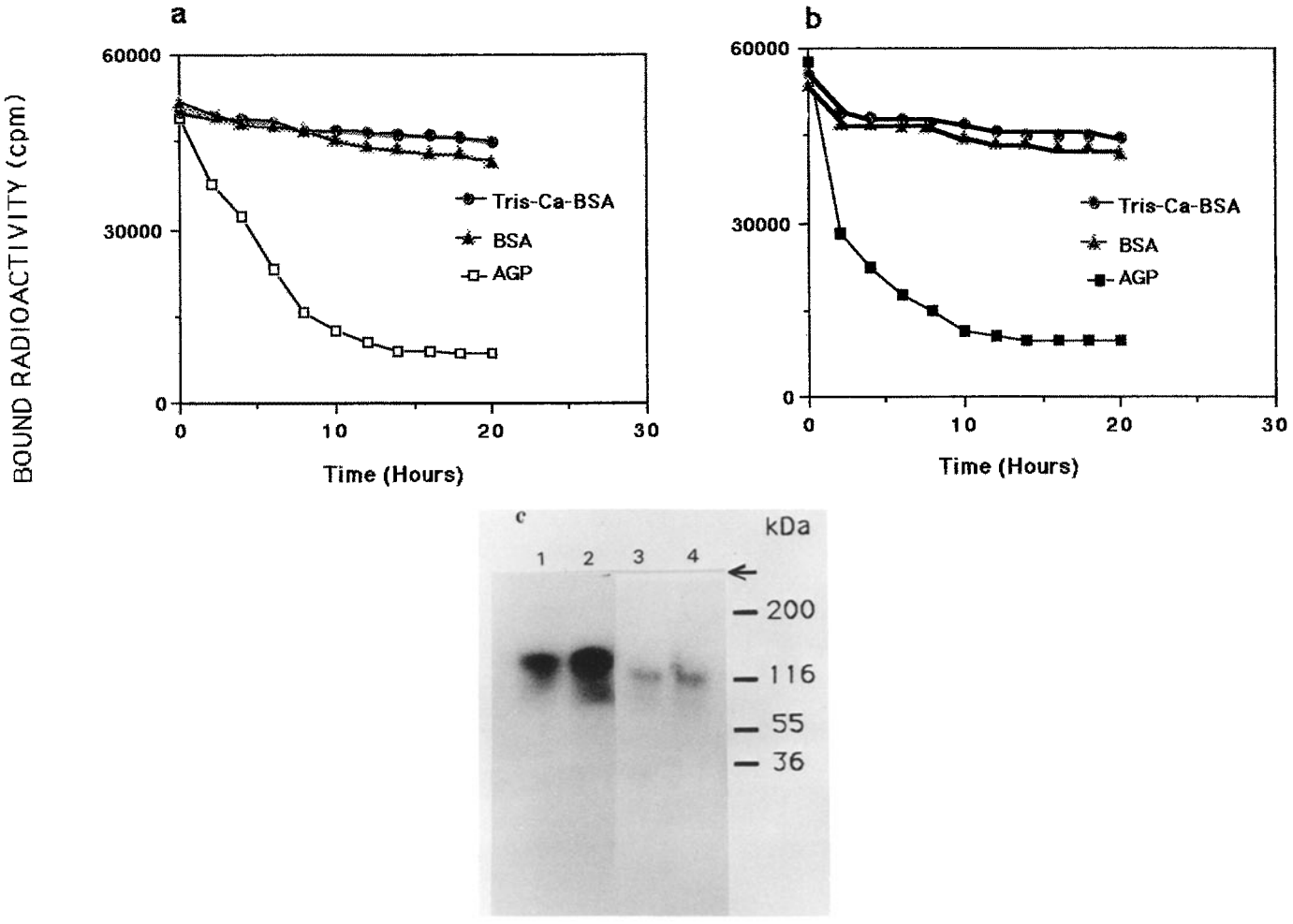

Figure 4. Reversion of rgp160 or rgp120 binding to AGP-agarose. (**a**) Reversion of [^{125}I]rgp160 binding to AGP-agarose by AGP; matrix-bound rgp160 was incubated at 37°C for 2–20 h with AGP (20 mg ml^{-1}); Tris-Ca-BSA 0,05% and BSA (20 mg ml^{-1}) were used as controls. (**b**) Reversion of [^{125}I]rgp120 binding to AGP-agarose by AGP as described above. (**c**) SDS-PAGE and autoradiography of rgp160 and rgp 120 after and before specific elution from AGP-agarose by AGP: rgp 160 eluted by AGP (lane 1), original rgp160 (lane 2), rgp120 eluted by AGP (lane 3), original rgp120 (lane 4).

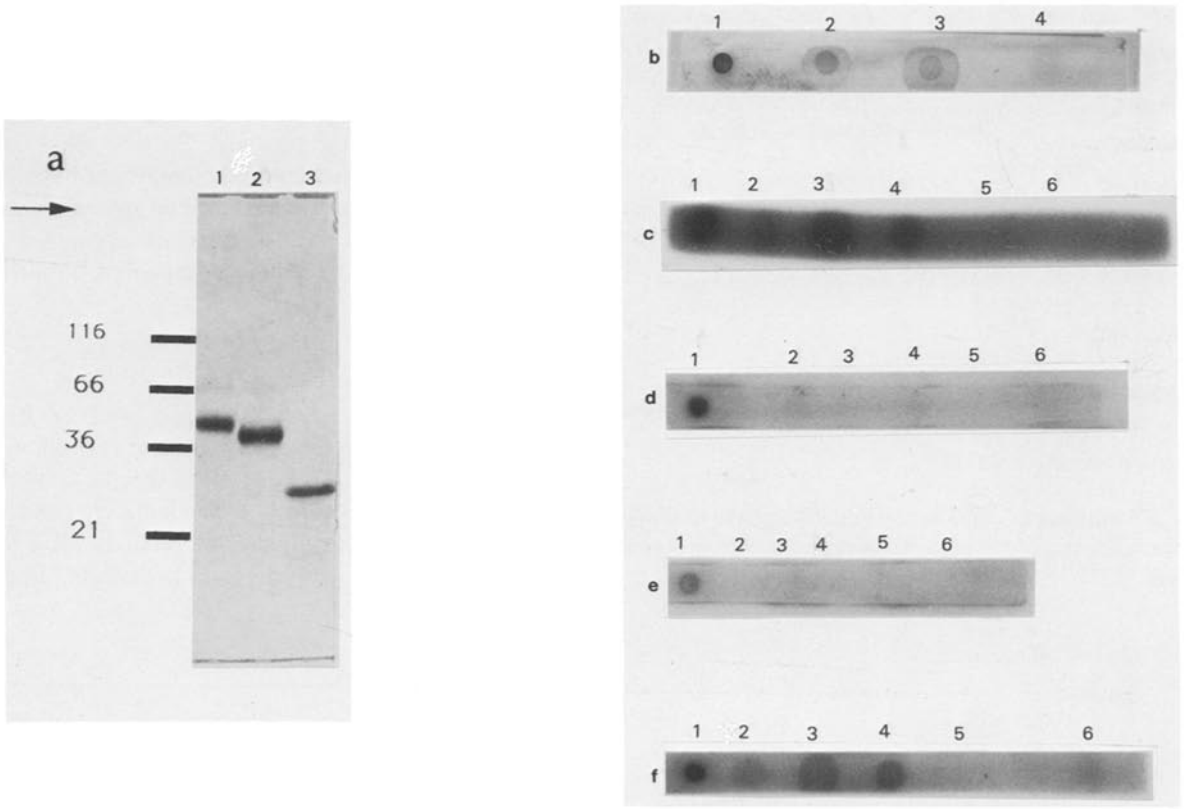

Figure 5. Dot spot analysis of the interaction of native, desialylated or deglycosylated AGP with [^{125}I]rgp160: (**a**) SDS-PAGE and Coomassie staining of native (1), desialylated (2) or deglycosylated AGP (3). (**b**) Interaction of native (2), desialylated (3) or deglycosylated AGP (4) with peroxydase-labelled Con A. Con A alone (1). (**c**) Autoradiography of rgp160 interaction with Con A at 2 μg (1), native AGP (2 μg) (2 and 3), desialylated AGP at 2 μg (4), deglycosylated AGP (2 μg) (5), PNA (2 μg) (6). Effect of rgp160 preincubation with 2 mg ml^{-1} of mannan (**d**), AGP (**e**) or β-D-glucan (**f**) on its binding to AGP; dot number as for (c).

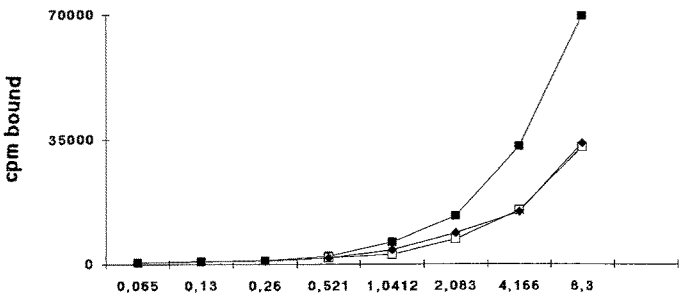

Figure 6. (■) Effect of [^{125}I]rgp160 concentration (M) on binding of [^{125}I]rgp160 to the MDM (cpm); (□): effect of AGP (25 μM), mannan at 2 mg ml^{-1} (◆) on the binding of rgp160 to the MDM.

Table 2. [^{125}I]rgp160 binding to monocyte-derived macrophages (MDM).

	Bound radioactivity (cpm)	*% inhibition*
Control	4720 ± 1450	
AGP (25 μM)	2050 ± 900*	57 ± 10
Mannan (2 mg ml^{-1})	2685 ± 1145*	45 ± 17
Dextran (2 mg ml^{-1})	3860 ± 1275	16 ± 16
β-d-glucan (2 mg ml^{-1})	3970 ± 1015	10 ± 5

Monocyte-derived macrophages (MDM) were purified and cultured as described in Materials and methods. [^{125}I]rgp160 was preincubated with AGP, mannan or β-D-glucan for 1 h at 37°C prior to incubation with the cells for 3 h at 4°C. Results are means ± SD of three independent experiments. Statistical significance of the difference relative to controls are determined by the paired Student's *t* test: *p<0.05.

HIV-1 envelope glycoproteins by showing that rgp160 and rgp120 can also recognize the mannosyl and *N*-acetylglucosaminyl residues present on AGP, a protein having a complex *N*-linked glycan structure, as already reported for other proteins [31, 32].

The binding of rgp160/rgp120 to AGP-agarose is inhibited by mannan, an oligomannose structure based on a backbone of repeating [1–6] linked α residues with short 1-2 and 1-3 linked α-mannose side chains [33], and by the neoglycoproteins β-D-GlcNAc$_{47}$-BSA and α-D-Man$_{20}$-BSA. Both AGP,

with complex type glycans containing galactose, fucose and sialic acid residues in addition to mannose and *N*-acetylglucosamine [34], and fetuin which contains 20% *O*-linked glycans and 80% *N*-linked complex type glycans [35, 36], also inhibited rgp160/rgp120 binding to AGP-agarose. In addition, binding of rgp160 to the matrix was inhibited (>50%) by complex-type asialo-agalacto tetraantennary oligosaccharide of AGP and by oligomannose 9 from porcine thyroglobulin.

In contrast, there was no inhibition with monosaccharides such as Man, D-Gal, GlcNAc, Me-α-Glc, Me-α-Gal or β-D-glucan, which indicates that the observed binding property could be specific for oligosaccharides. Complex-type fucose-substituted asialo-agalacto biantennary core from porcine thyroglobulin and complex-type asialo-galactosylated triantennary oligosaccharide from bovine fetuin had a limited effect which suggests that the differences in sugar and the type of branching of oligosaccharides are involved in their interaction with rgp120/160. Furthermore, AGP and asialo-AGP had similar inhibitory effects on rgp160 binding to AGP-agarose, whereas deglycosylation of AGP by endo F/N glycanase, which cleaves all glycans *N*-bonded to asparagine, totally abrogated AGP binding to rgp160. These results strongly implicate the carbohydrate moieties of AGP as the ligand recognized by rgp160. Because fetuin, AGP and asialo-AGP had comparable effects, interaction of rgp160 with sialyl or galactosyl structures of *O*- or *N*-linked glycans can be excluded. The mannosyl specificity of rgp160/120 interactions with AGP-agarose was also indicated by reversion studies which showed that the majority (about 85%) of radiolabelled molecules were specifically retained on the affinity matrix and eluted by mannan in both cases.

We may then conclude that rgp160/120 specifically recognize GlcNAc/Man residues present on AGP in an internal position. However, the affinity of rgp160/120 for AGP is weak because the C_{50} values obtained were in the μM range (25 and 28 μM, respectively). Nevertheless, these C_{50} are of the same order as those reported by other investigators for other mannose-binding proteins [31, 32].

Variation in glycosylation has been shown for different hepatic glycoproteins [37]. Extensive studies have been performed on the glycans present on AGP fractionated by affinity chromatography on Con A-Sepharose [14, 25, 38]. They have shown the absence of biantennary glycans on AGP A and the presence of one and two biantennary glycans on AGP B and AGP C, respectively, tri- and tetra-antennary chains occupying the remaining glycosylation sites. Bennett and Schmid [39] have shown that the oligosaccharide structures present on AGP determine the effectiveness of its immune suppressive properties, AGP C containing a high degree of biantennary structures having the lowest activity level [40]. Therefore, the degree of branching might imply that properties of each form of AGP are different. Because of carbohydrate involvement in AGP-rgp160 binding, it was therefore of interest to learn which of these types of branching are involved in this binding. However, in the present study, the three variants had the same inhibitory effect on AGP-rgp160 interaction.

None of these carbohydrate ligands bound rgp120 or rgp160 at 'physiological' Ca^{2+} concentration. This can be explained by the role of calcium in oligomerization of HIV-1 *Env* glycoproteins [41, 42]. Therefore, the hypothesis that Ca^{2+} treated rgp 120 or rgp160 mimics the behaviour of HIV-1 extra-membranous *Env* glycoprotein associated to the envelope lipid bilayer can be considered.

By a different experimental approach, with MDM cells, we confirm the specific interaction of AGP with rgp160. Only preincubation of rgp160 with AGP or with mannan significantly diminished rgp160 binding to the cells, while pretreatment of the cells with AGP was inefficient. This suggests that AGP *N*-linked glycan interactions with rgp120/160 can modulate free HIV-1 *Env* glycoprotein binding to $CD4^+$ monocytic cells. However, AGP or mannan had no effect on binding of [^{125}I]rgp160 to the $CD4^+$ lymphoïd cell line CEM, which strongly suggests that HIV-1 *Env* glycoprotein does not bind in a similar way to monocytic or lymphoïd cells. In accordance with this concept, previous studies [29, 43] have shown that anti-Leu 3a, an anti-CD4 monoclonal antibody known to interfere with gp120/CD4 binding [44] significantly reduced gp160 binding to CEM cells but had no effect on gp160 binding to MDM under similar conditions. In conclusion, the present study opens the possibility of interactions between AGP and free virus or circulating HIV-1 glycoproteins. This hypothesis is currently under investigation in our laboratory.

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References

1. Kieny MP, Lathe R, Riviere Y, Dott K, Girard M, Montagnier L, Lecoq J P (1988) *Protein Eng* **2**: 219–25.
2. Gelderblom HR (1991) *AIDS* **5**: 617–38.
3. Abel CA, Noble EV, Raymond WW, Mielke CH, Klock JC (1987) *Fed Proc* **46**: 1318–21.
4. Mizuochi T, Spellman JW, Larkin M, Solomon J, Basa LJ, Feizi T (1988) *Biochem J* **254**: 599–605.
5. Geyer H, Holschbach C, Hunsmann G (1988) *J Biol Chem* **263**: 11760–67.
6. Fenouillet E, Clerget-Raslain B, Gluckman JC, Guetard D, Montagnier L, Bahraoui E (1989) *J Exp Med* **169**: 807–22.
7. Gruters RA, Neefjes JJ, Tersmotte M, De Goede Rey, Tulp A, Huisman HG, Miedema F, Ploegh HL (1987) *Nature* **330**: 74–77.
8. Pal R, Hoke GM, Sarngadharan MG (1989) *Proc Natl Acad Sci* USA **86**: 3384–88.

9. Gattegno L, Sadeghi H, Saffar L, Bladier D, Clerget-Raslain B, Gluckman J C, Bahraoui E (1991) *Carbohydr Res* **213**: 73–79.
10. Haïdar M, Seddiki N, Gluckman J C, Gattegno L (1992) *Glyconjugate J* **9**: 315–323.
11. Schmid K (1953) *J Am Chem Soc* **75**: 60–68.
12. Schmid K, Kaufman H, Isemura S, Bauer F, Emura J, Motoyama T, Ishiguro M, Nanno S (1973) *Biochemistry* **12**: 2711–24.
13. Schmid K, Nimberg RB, Kimura A, Yamaguchi H, Binette JP (1977) *Biochem Biophys Acta* **492**: 291–302.
14. Bayard B, Kerckaert JP (1980) *Biochem Biophys Res Comm* **95**: 777–84.
15. Krusius T, Finne J, Rauvala H (1976) *FEBS Lett* **71**: 117–20.
16. Ogata S, Muramatsu T, Kobata A (1975) *J. Biochem* **78**: 687–96.
17. Koj A, Dubin A, Kasperczyk H, Bereta J, Gordon A H (1982) *Biochem J* **206**: 545–53.
18. Durand G (1989) *Prog Clin Biol Res* **300**: 247–63.
19. Meijer D F K, Van der Sluijs P (1989) In *Progress in Clinical and Biological Research. Alpha-1 Acid Glycoprotein* (Baumann P, Eap CB, Muller WE, Tillement JP, eds) pp. 143–67, New York: Alan R Liss.
20. Kremer JMH, Wilting J, Wilting JLHM (1988) *Pharmacol Rev* **40**: 1–12.
21. Pimpaneau V, Midoux P, Durand G, de Baetselier P, Monsigny M, Roche AC (1989) *Glycoconjugate J* **6**: 561–74.
22. Wain-Hobson S, Vartanian J, Henry M, Chenciner N, Cheynier R, Delassus S, Pedroza Martins L, Sala M, Nugeyre MT, Guetard D, Klatzmann D, Gluckman JC, Rozembaum W, Barre-Sinoussi F, Montagnier L (1991) *Science* **252**: 961–65.
23. Warren L (1959) *J Biol Chem* **234**: 1071–75.
24. Laemmli UK (1970) *Nature (London)* **227**: 680–85.
25. Bierhuizen MFA, De Wit M, Govers CARL, Ferwerda W, Koeleman C, Pos O, Van Dijk W (1988) *Eur J Biochem* **175**: 387–94.
26. Valentin A, Van Gergefelt A, Matsuda S, Nilsson K, Asjö B (1991) *J Acq Immun Defic Syndrome* **4**: 751–59.
27. Jouault T, Chapuis F, Olivier R, Parravicini C, Bahraoui E, Gluckman *JC AIDS* **3**: 125–33.
28. Horejsi V, Matousek M, Ticha T, Trnka T (1985) In *Lectins* (Bog-Hansen T C, ed.) pp. 298–306, Berlin: Walter de Gruyter.
29. Seddiki N, Ramdani A, Saffar L, Portoukalian J, Gluckman JC, Gattegno L (1994) *Biochem Biophys Acta* (in press).
30. Drickamer K (1987) *Kidney Int* **32** (suppl. 23): 67–74.
31. Maynar Y, Baenziger JU (1982) *J Biol Chem* **257**: 3788–94.
32. Childs M A, Feizi T, Yuen C, Drickamer K, Quesenberry (1990) *J Biol Chem* **265**: 20770–77.
33. Nakajima T, Ballou CE (1975) *Biochem Biophys Res Comm* **66**: 870–79.
34. Montreuil J (1984) *Biol Cell* **51**: 115–32.
35. Green ED, Edelt G, Baenziger JU, Wilson S, Van Halbeek H (1988) *J Biol Chem* **263**: 18253–68.
36. Cumming DA, Hellerquist CG, Harris-Brandts M, Michnick SW, Carver JP, Bendiak B (1989) *Biochimistery* **28**: 6500–12.
37. Hatton MWC, Marz L, Regoeczi E (1983) *Trends Biochem Sci* **92**: 287–91.
38. Perkins SJ, Kerckaert JP, Loucheux-Lefebvre MH (1985) *Eur J Biochem* **147**: 525–31.
39. Bennett M, Schmid K (1980) *Proc Natl Acad Sci USA* **77**: 6109–13.
40. Bories PN, Feger J, Benbernou N, Rouzeau JD, Agneray J, Durand G (1990) *Inflammation* **14**: 315–23.
41. E Mbemba, JA Czyrski, Gattegno L (1992) *Biochim Biophys Acta* **1180**: 123–29.
42. M Haïdar, JC Gluckman, Gattegno L (1994) *Glycoconjugate J*, (in press).
43. Finbloom DS, Hoover TL, Meltzer MS (1991) *J Immunol* **146**: 1316–21.
44. Klatzmann DR, McDougal JS, Maddon PJ (1990) *Immuno Defic Rev* **2**: 43–60.