**Characterization of the Serological Cross-reactivity between Glycoproteins of the Human Immunodeficiency Virus and Equine Infectious Anaemia Virus**

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**SUMMARY**

The reported serological relatedness between the major glycoproteins of human immunodeficiency virus (HIV gp120) and equine infectious anaemia virus (EIAV gp90) was examined using purified antigens in radioimmunoprecipitation (RIP), radioimmunoassay (RIA) and immunoblot assays with reference serum from acquired immunodeficiency syndrome (AIDS) patients, an anti-gp120 goat serum and EIAV-infected horse serum. To assess the contributions of glycoprotein oligosaccharide and peptide components to any observed reactivities, antigens treated with endoglycosidase F to remove carbohydrate were assayed in parallel with the intact glycoprotein. The results of the experiments indicated that the reactivity observed for each antigen was dependent on the immunoassay employed. The RIP and RIA analyses demonstrated that HIV gp120 is equally reactive with the AIDS patient serum, the goat anti-gp120 serum and the EIAV-infected horse serum, whereas the EIAV gp90 reacted only with the horse serum. In immunoblot assays, the HIV gp120 reacted with the AIDS patient serum, but not with the EIAV-infected horse serum. Deglycosylation of the HIV gp120 evidently increased its reactivity with the AIDS patient serum, had no significant effect on its reactivity with the goat antiserum, and essentially abolished its reactivity with the EIAV reference serum. Thus, it appears that the serological cross-reactivity observed between HIV gp120 and sera from EIAV-infected horses can be attributed to the oligosaccharide rather than the peptide components of the viral glycoprotein. These studies also emphasize the necessity of employing several assay procedures in assessing lentivirus antigenicity.

Animal lentiviruses are becoming increasingly important as models for examining the molecular biology of human immunodeficiency virus (HIV) and for evaluating treatment and vaccine procedures for acquired immunodeficiency syndrome (AIDS) in humans. The lentivirus subfamily of retroviruses currently contains visna virus of sheep, caprine arthritis–encephalitis virus, equine infectious anaemia virus (EIAV), simian immunodeficiency virus (SIV), and HIV. Recent isolates of feline T lymphotropic virus (Pedersen et al., 1987) and bovine immunodeficiency virus (BIV) (Gonda et al., 1987) evidently will also be included in the lentivirus subfamily. The classification of these viruses is based primarily on similarities of virion morphology, genomic organization and gene sequences, the role of macrophages/monocytes as host cells, and patterns of pathogenesis.

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Fig. 1. Analyses of HIV gp120 reactivity in RIP assays. Samples of purified $^{125}$I-labelled HIV gp120, either intact (a) or deglycosylated (b) were incubated with various test sera, and the resultant immune complexes recovered by Sepharose-bound Protein A and analysed by SDS-PAGE (Montelaro et al., 1984b). The test sera were (lanes 1) normal human serum, (lanes 2) pooled AIDS patient serum, (lanes 3) normal goat serum, (lanes 4) goat anti-HIV gp120 serum, (lanes 5) normal horse serum and (lanes 6) EIAV-infected horse serum. Lanes 7 and 8 are reference samples of the intact and deglycosylated $^{125}$I-labelled gp120, respectively.

Interestingly, reports of serological relatedness among the various lentiviruses have been limited, although the identification of cross-reactive antigens would be important in identifying highly conserved protein sequences to serve as candidates for diagnostic or vaccine purposes. Serological cross-reactivities have been reported between the major core proteins of HIV (p24) and the analogous proteins of SIV (p26) (Murphey-Corb et al., 1986), EIAV (p26) (Montagnier et al., 1984; Goudsmit et al., 1986) and BIV (p26) (Gonda et al., 1987) employed in radioimmunoprecipitation (RIP), radioimmunoassay (RIA) or immunoblot procedures with serum from infected patients and animals. In addition, Schneider et al. (1986) recently reported that HIV gp120 could be precipitated with sera from EIAV-infected horses, but not with sera from uninfected animals. This observation raises the possibility of conserved epitopes between EIAV and HIV glycoproteins, perhaps analogous to the shared ‘interspecies-specific’ determinants identified on murine, feline and simian oncoviruses (Schneider & Hunsmann, 1978).

Several reports have indicated that the pattern of serological reactivity observed with lentiviruses is dependent on the immunoassay employed (Montagnier et al., 1984; Sarngadharan et al., 1985; Goudsmit et al., 1986). Thus, we performed a series of immunoassays (RIP, RIA and
immunoblot) to ascertain the reactivity of the respective purified surface glycoproteins of HIV (gp120) (Robey et al., 1986) and EIAV (gp90) (Parekh et al., 1980) against a panel of sera including an AIDS patient serum pool (Robey et al., 1986), a goat serum raised against purified HIV gp120 (Robey et al., 1986), and reference sera from horses naturally infected with EIAV (Montelaro et al., 1984a, b). In each case, serum samples from an uninfected source were included as a control.

Previous studies with lentiviruses have indicated that RIP is the most sensitive procedure for detecting serological cross-reactivity (Allan et al., 1985; Goudsmit et al., 1986). Purified $^{125}$I-labelled HIV gp120 and EIAV gp90 were evaluated in RIP assays using the sera cited above and standard procedures (Montelaro et al., 1984b; Robey et al., 1986). The resultant immune precipitates were analysed by SDS-PAGE and autoradiography to assay the extent of antigen precipitation by each serum sample. Representative patterns of reactivity of HIV gp120 and EIAV gp90 obtained in RIP assays are presented in Fig. 1(a) and Fig. 2(a), respectively. The results (Fig. 1a) demonstrate that HIV gp120 was strongly precipitated by the AIDS patient serum, the goat anti-HIV gp120 serum and the EIAV-infected horse serum. The EIAV gp90 (Fig. 2a) was effectively precipitated only by the EIAV-infected horse serum; neither the AIDS patient serum nor the goat anti-gp120 serum precipitated significant amounts of EIAV gp90. Moreover, none of the control sera precipitated HIV gp120 or EIAV gp90. These observations confirm the previous report of HIV gp120 reactivity with EIAV-infected horse serum

Fig. 2. Analyses of EIAV gp90 reactivity in RIP assays. Samples of purified $^{125}$I-labelled EIAV gp90, either intact (a) or deglycosylated (b) were analysed in RIP assays as described in Fig. 1. Lanes 1 to 6 as in Fig. 1. Lanes 7 and 8 are reference samples of intact and deglycosylated $^{125}$I-labelled EIAV gp90, respectively.
(Schneider et al., 1986) and extend the analysis to reveal, for the first time, that the cross-reactivity is only one way, i.e. AIDS patient and goat anti-gp120 sera fail to precipitate EIAV gp90. This unidirectional cross-reactivity is reminiscent of the observation that EIAV-infected horse sera precipitate HIV p24, but AIDS patient sera fail to precipitate EIAV p26 (Montagnier et al., 1984; Sarngadharan et al., 1985; Goudsmit et al., 1986).

To ascertain the relative contributions of the carbohydrate and peptide components of the viral glycoproteins to the observed serological reactivities, samples of ¹²⁵I-labelled purified HIV gp120 and EIAV gp90 were digested with endoglycosidase F to remove oligosaccharides from the polypeptide (Elder & Alexander, 1982) before assay in RIP procedures. The deglycosylated HIV gp120 (Fig. 1 b) was precipitated by the AIDS patient serum and the goat anti-gp120 serum. In contrast, deglycosylation of the HIV gp120 completely inhibited its reactivity with the EIAV-infected horse serum. These results indicate that the predominant antibody populations monitored by RIP assays are specific for the HIV gp120 peptide chain in the case of AIDS patient and goat anti-gp120 sera, but apparently oligosaccharide-specific in the case of EIAV-infected horse serum. The data in Fig. 2(b) also demonstrate that the deglycosylated EIAV gp90 was still precipitated efficiently by the EIAV-infected horse serum, confirming results obtained previously by competitive RIA analyses in which the predominant antigenicity was assigned to gp90 peptide sequences (Montelaro et al., 1984b).

Thus, the RIP data indicate that the observed reactivity of HIV gp120 with EIAV-infected horse serum can be attributed to antibodies that are generated during EIAV infection and which depend on gp120 oligosaccharide determinants for binding.

To obtain a more quantitative measure of glycoprotein antigenicity, HIV gp120 and EIAV gp90 reactivities were also evaluated using standard RIA procedures (Montelaro et al., 1984b; Robey et al., 1986) and the panel of sera described above. The data in Fig. 3(a) demonstrate
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Fig. 4. Analyses of HIV gp120 and EIAV gp90 by immunoblot assays using either EIAV-infected horse serum (a) or pooled AIDS patient serum (b). In each panel, lane 1 contains intact EIAV gp90, lane 2 contains deglycosylated gp90, lane 3 contains intact HIV gp120, and lane 4 contains deglycosylated gp120. Procedures for SDS-PAGE of viral antigens, transfer to nitrocellulose, incubation with sera and 125I-labelled Protein A, and for autoradiography have been described previously (Montelaro et al., 1984a).

virtually identical patterns of reactivity for the intact and deglycosylated HIV gp120 with the goat antiserum in RIA. In contrast, deglycosylation of gp120 apparently increased its reactivity with the pooled AIDS patient serum by about twofold relative to intact gp120 (Fig. 3b). Most striking is the observation that deglycosylation of HIV gp120 reduced its reactivity with the EIAV-infected horse serum by at least 90% (Fig. 3c). The reactivity profiles of intact and deglycosylated EIAV gp90 (Fig. 3d) with the EIAV-infected horse serum reveals about a 35% diminution of reactivity caused by the removal of gp90 oligosaccharides. These results agree with those obtained in the RIP analyses above and indicate that HIV gp120 oligosaccharides mediate virtually all of the glycoprotein reactivity with the immune horse serum. In contrast, it appears that the gp120 oligosaccharides may actually inhibit binding of the antibody found in AIDS patient sera.

Samples of purified HIV gp120 and EIAV gp90, both intact and deglycosylated, were also assayed for reactivity with the EIAV reference serum and the AIDS patient serum in immunoblot analyses after transfer to nitrocellulose following SDS-PAGE (Montelaro et al., 1984a). The autoradiogram in Fig. 4(a) lanes 1 and 2 indicates that both the intact and deglycosylated HIV gp120 are reactive with AIDS patient sera in the immunoblot assay. In fact, the deglycosylated gp120 was reproducibly more reactive than the intact glycoprotein, as observed in the RIA procedures described above. Interestingly, the EIAV reference serum (Fig. 4b, lanes 1 and 2) displayed no reactivity with either the intact or deglycosylated HIV gp120 in the immunoblot assay. The absence of reactivity in immunoblots between gp120 and the EIAV reference serum contrasts with the results of RIP and RIA procedures and emphasizes the need to employ a variety of assays in assessing lentivirus antigenicity.

Intact and deglycosylated EIAV gp90 failed to react with the AIDS patient serum in the immunoblot assay (Fig. 4a, lanes 3 and 4). In contrast, both intact and deglycosylated gp90 reacted with the EIAV reference serum in the immunoblot assay (Fig. 4b, lanes 3 and 4), with the deglycosylation causing some reduction in antibody reactivity relative to that of the intact glycoprotein. Thus the results of immunoblot assays of gp90 apparently correspond to the patterns of antigenicity observed for gp90 in RIA and RIP assays.

The finding of HIV gp120 carbohydrate-dependent antibody in EIAV-infected horse serum raises a question of whether this antibody population is capable of neutralizing HIV. To answer
this question, the neutralization capacities of the EIAV reference sera against several HIV isolates were analysed in parallel using previously described assay procedures (Robey et al., 1986). The results of this experiment indicated that under conditions in which the AIDS patient serum and goat anti-gp120 serum neutralize HIV, the EIAV-infected horse serum had no apparent effect on HIV infectivity.

In conclusion, the studies described here demonstrate that the serological reactivity observed between HIV gp120 and EIAV-infected horse serum can be attributed to non-neutralizing antibodies whose binding is dependent on the oligosaccharide components of the viral glycoprotein. The most likely explanation of this specificity is that the antibodies in the equine immune serum are actually specific for, and bind to, gp120 carbohydrate chains. However, the results presented here cannot completely exclude the possibility that the horse serum recognizes non-linear protein determinants on gp120 that are held in place by the presence of oligosaccharide chains. In support of the first model is the fact that EIAV gp90 and HIV gp120 lack significant $env$ sequence homology, even though they display remarkably similar structural features (Rushlow et al., 1986). The latter model may be supported by the observation that HIV gp120 reactivity with AIDS patient sera is significantly altered by changes in glycoprotein conformation, as observed when comparing non-denaturing and denaturing conditions, and reducing and non-reducing assay conditions (W. G. Robey, unpublished data). We have previously been able to distinguish between these two possibilities in assaying the contribution of oligosaccharides to EIAV gp90 antigenicity by examining the ability of purified gp90 oligosaccharides to compete with intact gp90 for antibody in RIA (Montelaro et al., 1984b). Similar experiments with HIV gp120 are currently not feasible because of the inability to obtain sufficient quantities of this glycoprotein for preparation of the carbohydrate components.

On the basis of existing comparative serological and gene sequence data, it is our opinion that interspecies determinants in lentiviruses are confined to the $gag$ and $pol$ proteins and that the $env$ proteins are highly specific for each virus. The studies described here also emphasize the variation in serological reactivity patterns obtained with different immunoassay procedures and the need to perform a variety of assays in surveying the serological properties of the lentiviruses.

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