Thiol-Selective Mechanism of HIV Antigen Conjugation with Serum IgM, IgG, and IgA

V. V. Kostyushov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 12, pp. 627-629, December, 2000
Original article submitted May 28, 1999

The effects of HIV antigen glycoproteins gp160, gp41, and gp36 on thiol-dependent specific (affinity) binding of serum IgM, IgG, and IgA with the corresponding antigens were determined by the formation of signal thiol-containing analytes (free nonprotein SH groups). Free nonprotein SH groups were not found in the reaction mixture, which indicated that HIV antigen glycoproteins blocked this process. The results suggest that the thiol-selective mechanism underlies *in vitro* conjugation of HIV antigen glycoproteins gp160, gp41, and gp36 with serum immunoglobulins. Previous studies showed that this mechanism of conjugation with immunoglobulins is not characteristic of other lymphotropic viruses (*e.g.*, hepatitis B virus).

Key Words: *HIV antigen; glycoproteins; nonprotein SH groups; immunoglobulins; thiol-selective mechanism*

Experimental and clinical data demonstrate an important role of thiol-dependent affine binding (TDAB) of serum IgM, IgG, and IgA with monospecific sera *in vitro* [1,2]. This universal mechanism underlying specific (affine) binding of the antigen and antibody is realized via the formation of thiol-containing analytes (free nonprotein SH groups) in deproteinized reaction mixtures.

In HIV-infected patients, TDAB of serum immunoglobulins with the corresponding antigens is impaired, which is confirmed by the absence of free nonprotein SH groups in the reaction mixture.

We hypothesized that HIV glycoproteins are conjugated with IgM, IgG, and IgA by a thiol-selective mechanism [3] and block genetically determined TDAB of immunoglobulins with the corresponding antigens. This assumption is confirmed by the fact that SH and SS groups play an important role in molecular mechanisms of structural and conformational organization and functional activity of specific proteins (*e.g.*, immunoglobulins) in various biological reactions [4,6].

Department of Pharmacology, Odessa State Medical University

Here we studied the thiol-selective mechanism underlying *in vitro* conjugation of HIV antigen (AgHIV) glycoproteins gp160, gp41, and gp36 with serum immunoglobulins from healthy donors.

MATERIALS AND METHODS

Serum samples were taken from 25 healthy donors. We studied the interaction of IgM, IgG, and IgA with monospecific sera against IgM, IgG, and IgA (KONE C), AgHIV containing recombinant membrane glycoproteins gp160, gp41, and gp36 (Sanofi Diagnostics Pasteur), and hepatitis B virus surface antigen (HBsAg) comprising 3 recombinant HBsAg subtypes (Sanofi Diagnostics Pasteur). The reagents were prepared by routine methods. We performed 5 series of *in vitro* experiments.

In series I, we studied TDAB of serum immunoglobulins with anti-IgM, anti-IgG, and anti-IgA. These antigens were mixed 10:1 with serum samples. The following reaction mixtures were prepared: serum+anti-IgM, serum+anti-IgG, and serum+anti-IgA.

In series II and III, we studied the thiol-dependent mechanism underlying nonspecific interaction of proteins from a serum containing no anti-HBs and anti-

0007-4888/00/0012-1147$25.00

HIV with HBsAg and AgHIV, respectively. The corresponding antigens were mixed 10:1 with serum samples. The following reaction mixtures were prepared: serum+ HBsAg and serum+AgHIV.

In series IV and V, we studied the effects of HBsAg and AgHIV on TDAB of serum immunoglobulins with the corresponding antigens. Serum samples from healthy donors were pretreated with antigens (10:1) at 37°C for 60 min. Anti-IgM, anti-IgG, and anti-IgA were added to serum+HBsAg and serum+ AgHIV reaction mixtures in a 10:1 ratio.

All reaction mixtures were thermostated at 37°C for 180 min. The thiol-selective mechanism of AgHIV and HBsAg conjugation with immunoglobulins was determined by their effects on TDAB of serum immunoglobulins with the corresponding antigens. Specific binding was evaluated by the presence or absence of signal thiol analytes (free nonprotein SH groups) in the reaction mixture. These compounds were detected by amperometric titration with $AgNO_3$ after deproteinization. The measurements were performed on the 1st, 60th, 120th, and 180th min of incubation. The content of free nonprotein SH groups was expressed in μmol/liter.

The absence of HBsAg and antibodies to HIV in sera from healthy donors was confirmed using Monolisa HBsAg 2 Generation and Genelavia Mixt HIV 1+2 kits (Sanofi Diagnostics Pasteur). The results were analyzed using Primer Biostatistics and SigmaStat software.

RESULTS

Amperometric titration with $AgNO_3$ revealed no free nonprotein SH groups in deproteinized serum from healthy donors.

In series I, the interaction of serum immunoglobulins with anti-IgM, anti-IgG, and anti-IgA was accompanied by the formation of free nonprotein SH groups in all reaction mixtures (Table 1).

TABLE 1. Content of Free Nonprotein SH Groups (μmol/liter) in Deproteinized Reaction Mixtures

Mixture	Time of thermostatic treatment, min			
	1	60	120	180
Serum+anti-IgM	17.27	19.81	23.12	21.39
+HbsAg	15.68	16.79	13.20**	14.52*
Serum+anti-IgG	25.44	28.87	31.97	34.37
+HbsAg	23.47	25.00	28.53	30.88
Serum+anti-IgA	19.73	24.00	25.55	20.93
+HbsAg	18.21	19.16	20.77	17.34

Note. Free SH groups were not found in serum and serum+AgHIV+ IgM/IgG/IgA mixtures at all periods of incubation. *$p<0.01$ and **$p<0.05$ compared to serum+anti-IgM mixture.

In series II and III, the interaction of serum proteins with HBsAg and AgHIV was not accompanied by the formation of free nonprotein SH groups.

After pretreatment of serum samples from healthy donors with HBsAg, the content of free nonprotein SH groups in deproteinized serum+HBsAg+anti-IgG and serum+HBsAg+anti-IgA reaction mixtures did not differ from that in deproteinized serum+anti-IgG and serum+anti-IgA mixtures. Only in the serum+HBsAg+ anti-IgM mixture, the content of free nonprotein SH groups after 120- and 180-min incubation was much lower than in the serum+anti-IgM mixture ($p<0.05$ and $p<0.01$, respectively, Table 1).

After pretreatment of donor serum with AgHIV, no free nonprotein SH groups were found in reaction mixtures.

Thus, we revealed a thiol-selective molecular mechanism of *in vitro* interaction between AgHIV glycoproteins displaying selective immune toxicity and serum immunoglobulins from healthy donors. Before the addition of AgHIV glycoproteins to donor serum, specific (affinity) binding of IgM, IgG, and IgA with the corresponding antigens was always accompanied by the formation of free nonprotein SH groups in deproteinized reaction mixtures. AgHIV glycoproteins completely blocked these thiol-dependent mechanisms and, therefore, free nonprotein SH groups were absent. Probably, AgHIV glycoproteins completely block destabilization of mixed disulfide bonds between low-molecular-weight thiol-containing compounds and proteins. After pretreatment with AgHIV glycoproteins, these bonds become inaccessible for anti-IgM, -IgG, and -IgA. Therefore, low-molecular-weight thiol-containing compounds retain their preformed state, which contributes to impaired effector and regulatory functions of immunoglobulins in HIV-infected patients [5]. Probably, HIV abolishes presentation and recognition of determinant groups for anti-IgM, anti-IgG, and anti-IgA immunoglobulins. This thiol-selective interaction between immunoglobulins and AgHIV glycoproteins leads to their conjugation. In patients with asymptomatic HIV infection, the ratio of physiological and pathological processes probably depends on the formation, stability, and degradation of immunoglobulin-AgHIV glycoprotein conjugates, peculiarities of their structural and conformational organization, and biological properties. It cannot be excluded that conjugated immunoglobulins lose their immunogenic properties in relation to anti-IgM, anti-IgG, and anti-IgA, while AgHIV gains new antigen properties. This process contributes to antigenic mimicry of AgHIV and hinders etiological diagnostics of HIV infection by enzyme im-

munoassay, immune blotting, and polymerase chain reaction.

It can be hypothesized that clinical manifestation of AIDS is related to impaired thiol-selective mechanisms underlying conjugation of immunoglobulins with AgHIV glycoproteins and destabilization of these conjugates. These changes result in conversion of AgHIV from the preformed to active state.

This assumption agrees with clinical and immunological studies showing impairment of genetically determined TDAB of immunoglobulins with anti-IgM, anti-IgG, and anti-IgA in HIV-infected patients [3].

Our findings put in doubt on the primary role of lymphocyte damages with HIV. Experimental data indicate that the pathogenetic method proposed previously can be used for the diagnostics of HIV infection [3]. The elaboration of pathogenetic approaches holds much promise because of pronounced genetic variability of HIV and varying serologic, neutralizing, and phenotypic properties. Independently on the existence of serological variants and genetic variability of HIV, the thiol-selective mechanism always underlies its interaction with immunoglobulins. The pathogenetic method does not depend on antigenic mimicry of HIV and, therefore, is a promising diagnostic approach.

The thiol-dependent mechanism underlying conjugation of AgHIV glycoproteins with specific serum proteins is not characteristic of other lymphotropic viruses (*e.g.*, hepatitis B virus). These data confirm selective immune toxicity of AgHIV and should be taken into account in elaborating anti-HIV vaccine.

REFERENCES

1. V. V. Kostyushov, *Odes'kii Med. Zh.*, No. 6, 11-14 (1998).
2. V. V. Kostyushov, *Ibid.*, No. 1, 45-48 (1999).
3. V. V. Kostyushov, *Ibid.*, No. 2, 45-47 (1999).
4. Yu. M. Torchinskii, *Sulfur in Proteins* [in Russian], Moscow (1977).
5. R. M. Khaitov and B. V. Pinegin, *Immunologiya*, No. 1, 14-17 (1999).
6. E. S. G. Barron, *Adv. Enzymol.*, **11**, 201-206 (1951).