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Interaction of Human Apolipoprotein A-I with rsCD4 Receptor

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The interaction between recombinant soluble CD4 receptor (rsCD4) with human apolipoprotein A-I was studied by enzyme-linked immunosorbent assay. Our findings suggest that HIV-1 and human apolipoprotein A-I compete for the CD4 receptor and receptor for apoA-I.

Key Words: *CD4 receptor; human apolipoprotein A-I; env1; env2; HIV-1; HIV-2*

Cross-reactivity between HIV-1 surface proteins (env1) and antibodies against apolipoprotein A-I (apoA-I), and between antibodies against HIV-1 and human apoA-I [2,3] suggests that apoA-I can interacts with CD4 receptor. This interaction probably determines the antiviral effect of apoA-I on HIV-1 lysates [6,7]. The molecule of CD4 receptors is a marker of T helper lymphocytes. This molecule and major histocompatibility complex class II molecules recognize specific antigens and, therefore, play a role in the immune response. Here we studied the interaction between human apoA-I and CD4 receptor by enzyme-linked immunosorbent assay using individual and recombinant proteins.

MATERIALS AND METHODS

We used recombinant soluble CD4 receptor rsCD4 (Genentech) representing extracellular N-terminal fragment of human CD4 receptor that includes amino acid residues 1-367 [5]. This fragment is widely used in studies of the acceptor properties of this receptor. Surface HIV-1 proteins (env1, recombinant chimeric protein containing gp120 and gp41 peptides) were obtained from the Kapel' Company. Recombinant proteins containing gp110 and gp38 peptides were used as surface HIV-2 proteins (env2, Bioservis). Plasma samples from HIV-infected patients were obtained from State Research Center for Venereal Diseases (Vektor).

Monoclonal antibodies against human CD4 receptor (100 µl, dilution 1:50, Sorbent) were placed on a Costar plate for enzyme immunoassay and incubated at room temperature for 16 h. The plate was washed with phosphate buffered saline (PBS, pH 7.4) to remove unbound proteins and blocked with PBS, Tween, and blocking proteins (PBS-TP; 10% dry milk and 1% soluble *E. coli* protein) at 37°C for 1 h. rsCD4 (1 µg/ml, 100 µl) in PBS-TP was placed in wells, incubated at 37°C for 1 h, and titrated with surface HIV proteins or apoA-I. To this end, apoA-I (15-0.3 µg/ml, 100 µl) was added to wells and incubated at 37°C for 1 h. The plate was washed to remove proteins excess. Antibodies against apoA-I were placed in wells and incubated at 37°C for 1 h. Goat antibodies against rabbit IgG (secondary antibodies) labeled with horseradish peroxidase were added to visualize antibodies bound to apoA-I. The enzymatic reaction was performed with freshly prepared *o*-phenylenediamine (Merck) in a concentration of 20 mg per 100 ml 0.1 M phosphate-citrate buffer (pH 5.0) containing 0.006% H_2O_2. After 15 min the reaction was stopped by the addition of 50 µl 1 N H_2SO_4. Extinction was measured on a Multiscan Flow multichannel photometer at 492 nm. During

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titration of rsCD4 with surface proteins of HIV-1 or HIV-2, env1 or env2 (100 µl, 15-0.3 µg/ml) was placed in wells and incubated at 37°C for 1 h. The plate was washed to remove proteins excess. Antibodies against HIV-1 or HIV-2 were placed in wells and incubated at 37°C for 1 h. Goat antibodies against human IgG (secondary antibodies) labeled with horseradish peroxidase were added to visualize antibodies bound to env1 or env2. The enzymatic reaction was performed as described previously. Negative controls were run in parallel. Bovine serum albumin (BSA, 15-0.3 µg/ml, Sigma) was added to wells with sorbed rsCD4, incubated, and washed. Rabbit antibodies against BSA (Institute of Biochemistry) were added. Goat antibodies against rabbit IgG (secondary antibodies) labeled with horseradish peroxidase were added to visualize antibodies bound to BSA. The enzymatic reaction was performed as described previously.

The results of enzyme immunoassay were presented as titration curves, which reflected the dependence of optical density on the concentration of proteins used for titration of CD4 receptors on the plate.

RESULTS

The curves for titration of CD4 receptors with human apoA-I and env1 proteins are presented on Figure 1. Titration curve for human apoA-I lies below the corresponding curves for env1 proteins, which can be explained by lower affinity of apoA-I to the CD4 receptor (compared to env1 proteins). This, however, does not exclude competition of HIV-1 and human apoA-I for the CD4 receptor. This competition can underlie the antiviral effect of apoA-I. The competition between apoA-I and HIV-1 for the apoA-I receptor is of considerable interest. Our previous studies showed that complexes of apoA-I and reduced steroid hormones intensify gene expression and stimulate protein biosynthesis in various tissues [1,4]. HIV infection is accompanied by body weight loss. We hypothesize that this is associated with competition between apoA-I and HIV-1 for the apoA-I receptor.

Published data show that HIV-1 and HIV-2 produce similar effects on the same cells. Therefore, the interaction of CD4 receptors with HIV-1 and HIV-2 can be mediated by the same mechanisms. Our results

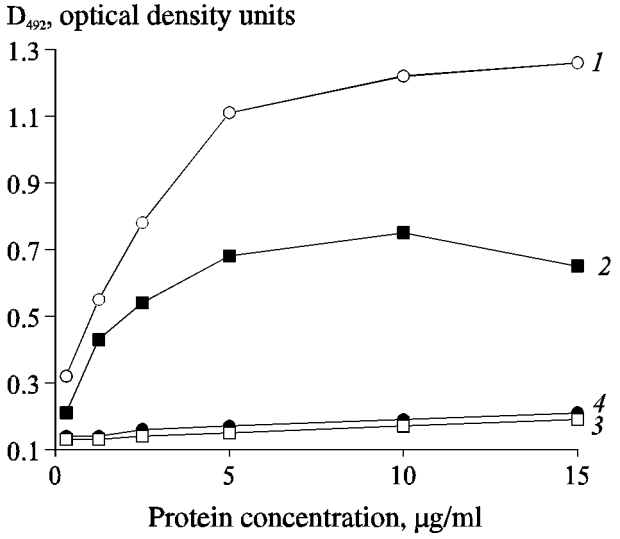

Fig. 1. Curves for titration of CD4 receptors with HIV-1 (*1*) and HIV-2 proteins (*3*) and apolipoprotein A-I (*2*). Negative control (*4*).

indicate that the interaction of HIV-1 (but not HIV-2) with lymphocytes and macrophages is realized via CD4 receptors. The results of titration of CD4 receptors on the plate with surface HIV-2 proteins did not differ from the negative control, which is probably related to the absence of their interaction. It cannot be excluded that the polypeptide chain of these recombinant HIV-2 proteins does not contain antigenic determinates for the CD4 receptor.

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