BIOPHYSICS AND BIOCHEMISTRY

Effect of L-Lysine-α-Oxidase on the Synthesis of Proteins Associated with Autoimmune Pathologies in HIV Infection

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In HIV-infected MT-4 cells, L-lysine-α-oxidase, an inhibitor of HIV reproduction, selectively inhibits the synthesis of histone proteins, including 18K antigen (H2B histone). This protein is the target for autoimmune suppression of helper T cells. The enzyme has a low effect on the synthesis of other proteins and DNA. In addition to 18K antigen, L-lysine-α-oxidase suppresses the synthesis of high-molecular-weight proteins (270, 220, 140, 100, 75, 70, and 60 kD).

Key Words: *L-lysine-α-oxidase; HIV infection; 18K antigen; protein synthesis*

In patients with AIDS, autoimmune disorders develop due to impaired regulation of synthesis and compartmentalization of cell proteins. For instance, the appearance of autoantibodies to 18K antigen [9], H2B histone exposed on the surface of HIV-infected cells [10], is an indicator of unfavorable prognosis for patients with AIDS. The development of classic autoimmune syndromes (nephritis, vasculitis, polymyositis, and myocarditis) has been documented in AIDS patients [5].

Our objective was to assess the rate and specificity of histone synthesis in cultured HIV-infected human T lymphocytes. The synthesis of 18K antigen was modified with L-lysine-α-oxidase (LO), an inhibitor of HIV reproduction *in vitro*.

MATERIALS AND METHODS

Lymphoblastoid MT-4 and Jurkat cells were grown in suspension in RPMI-1640 medium supplemented with 10% fetal calf serum and 100 U/ml gentamicin at 37°C in an atmosphere of 95% air/5% CO_2. MT-4 cells were infected with HIV (strain IIIB/H9) at a multiplicity of 0.02 TCD_{50} per cell. After 5 days, the cells were analyzed for the presence of HIV, using ELISA to evaluate cytopathic activity and expression of viral antigens [3].

The total histone fraction was isolated from the cells by extraction with 0.3 N HCl and electrophorezed as described [8]. Mice were immunized with H2B histone eluted from gel. The antigen was injected intraperitoneally three times at 1-week intervals.

Proteins and DNA were labeled with ^{35}S-methionine and ^{3}H-thymidine or ^{14}C-mixture of amino acids and analyzed as previously [1]. The radioactivity of the preparations was measured in a Marck-III spectrometer using a toluene scintillator. After electrophoresis, gel was dried and exposed onto an HS-II X-ray film (ORWO). The autoradiogram was compared with Coomassie R-250-stained gels.

RESULTS

In the first series of experiments, we examined the effect of LO on the synthesis of histones and 18K

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antigen (Fig. 1). In the concentration inhibiting HIV reproduction (70 ng/ml), LO practically did not affect the synthesis of DNA (Fig. 1, *a*) and proteins (Fig. 1, *b*) but inhibited by 20% the synthesis of histones, including 18K antigen (Fig. 1, *c*). Inhibition of the synthesis of 18K antigen was confirmed by ELISA with anti-H2B histone antisera (1:200). After 3 days of growth in the presence of LO, the light adsorbance of HIV-infected MT-4 cell culture was 2.5- to 2.8-fold lower than that of the control culture, indicating that LO modulates the synthesis of cell proteins which inhibit HIV reproduction.

In the second series, proteins labeled with ^{35}S-methionine were analyzed by autoradiography to confirm that LO modulates the synthesis of cell proteins. The synthesis of viral antigens was almost completely inhibited in the presence of this enzyme. It was found that LO changes the synthesis of cell proteins, particularly of high-molecular-weight proteins. L-Lysine-α-oxidase inhibited the synthesis of the major cellular protein (70 kD) and of some other proteins (Table 1).

On the other hand, within a 24-h period LO did not change the total protein production by cultured cells.

These findings indicate that antiviral activity of LO is mediated by a mechanism altering the synthesis of cell proteins which are probably involved in HIV reproduction. From comparison of these data with inhibition of the 18K antigen synthesis by LO we have concluded that these proteins may be responsible for the autoimmune suppression of cells induced by HIV infection.

While considering the possible mechanisms of the autoimmune manifestations occurring in HIV infection, it should be noted that viruses are regarded as the major causative agent of human autoimmune diseases [7]. Numerous studies show that viruses, including human T-lymphotropic retrovirus type I, are implicated in the etiology of a wide range of autoimmune diseases. Of special interest in this context is the observation that the occurrence of opportunistic viral infections (primarily of herpes virus infection) is rather high in HIV-infected individuals [6].

Several hypotheses based on the concept of molecular mimicry between cell proteins and viral antigens have been proposed to explain the virus-induced synthesis of autoantibodies [13]. Protein products of some HIV genes have antigenic determinants homologous to cell proteins, for example, to interleukin 2, α-thymosin, and immunoglobulins [12].

In T cells, CD4 is activated through interaction of CD4 receptors with processed antigenic fragments in conjunction with class II molecules of the major

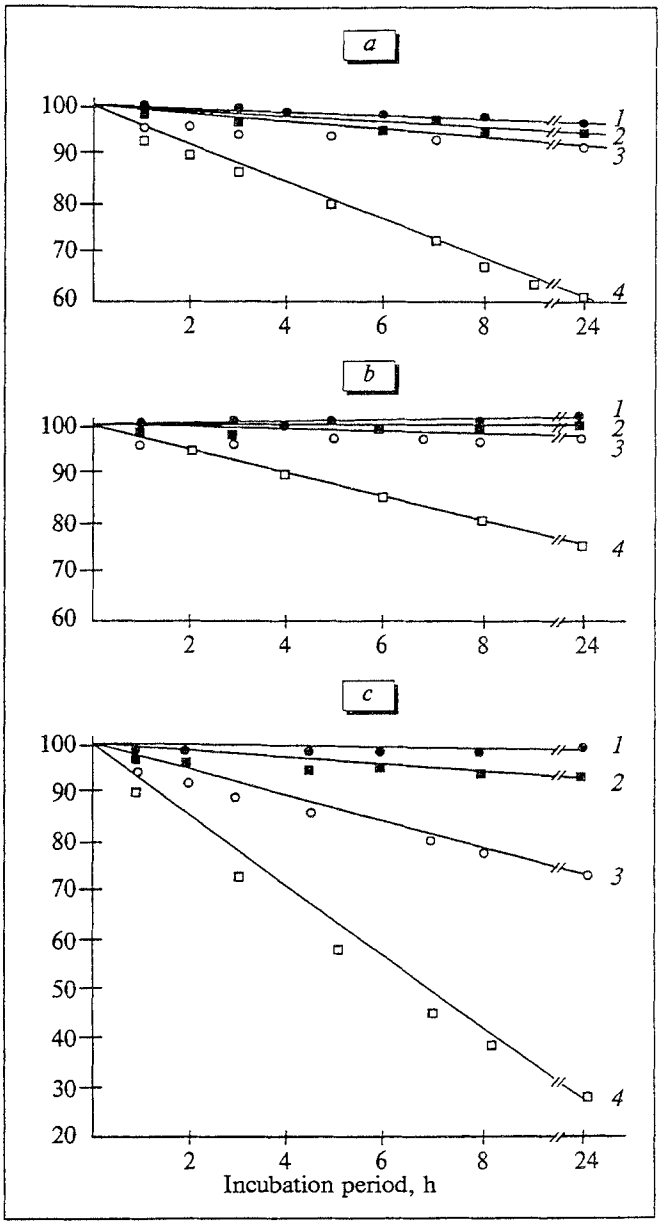

Fig. 1. Effect of the immunomodulator L-lysine-α-oxidase on the rate of DNA (*a*), total protein (*b*), and histone (*c*) synthesis in MT-4 cells cultured for 24 h. Ordinate: percentage ratio of label incorporations upon incubation with and without the immunomodulator in concentrations of 0.7 (*1*), 7 (*2*), 70 (*3*), and 700 (*4*) ng/ml.

histocompatibility complex (MHC) on the membranes of antigen-presenting cells. CD4 glycoproteins bind to MHC molecules, which increases the affinity of intercellular interactions and impairs the recognition of antigens by the low-affinity CD4 receptors [11]. It can be suggested that the disorders occurring in HIV-infected patients are associated with production of antibodies to the immunocompetent cells modifying the function of the T-cell CD4. A similar mechanism may operate in autoimmune diseases which are not associated with HIV infection.

TABLE 1. Molecular Weights and Electrophoretic Mobilities of Proteins Whose Synthesis Is Altered by the Immunomodulator L-Lysine-α-Oxidase

Protein No.	Relative electrophoretic mobility	Molecular weight, kD
1	0.013	270
2	0.038	220
3	0.076	140
4	0.150	100
5	0.210	75
6	0.230	70
7	0.280	60

Our results suggest that the development of autoimmunity at the initial stage of HIV infection is associated with enhanced synthesis of cell proteins required for HIV reproduction. This suggestion was confirmed by excessive production of H2B histone (18K antigen). Synthesis of this histone precedes the S phase of cell cycle [1,2]. Since the activation of replicative synthesis promotes incorporation of HIV genome into the host cell genome, the membrane expression of proteins with another compartmentalization in intact cell is quite possible. These proteins, including 18K antigen, subsequently become the sites of autoimmune pathology. This was confirmed by the finding of antihistone antibodies in systemic lupus erythematosus [4].

Whether viral etiology of autoimmune disease is proved or not, the investigation into the relationships between HIV infection and autoimmune disorders will be helpful for elucidation of clinical mechanisms and development of effective therapy of infectious diseases.

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