Identification of HIV-Specific Oligoclonal Immunoglobulins in Serum of Carriers of HIV Antibody

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Zone electrophoresis on agarose gel was performed on serum samples from HIV-antibody carriers and negative controls. Nitrocellulose strips precoated with an HIV preparation were then placed on top of the gels and developed by an immunoblotting procedure. A positive reaction was demonstrated between the HIV antigens and the HIV-antibody-positive serum samples with hypergammaglobulinemia and oligoclonal IgG bands. A negative reaction was found between the HIV antigens and HIV-antibody-negative serum samples from a normal person and a patient with monoclonal gammapathy. The presence of oligoclonal IgG bands in the serum of HIV-antibody carriers, and their positive identification with HIV antigens, indicates a specific immune response of the host to the HIV infection and supports the use of oligoclonal IgG bands as markers to follow the course of HIV infection.

Materials and Methods

Electrophoresis. In this study we used serum samples from asymptomatic persons who tested positive for HIV antibodies, first with an enzyme-linked immunosorbent assay (ELISA) and then with the Western blot test (Biotech Research Laboratories, Rockville, MD 20857). We also used serum samples from a healthy person and a patient with a

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strong monoclonal band that tested negative by the Western blot test.

Zone electrophoresis of serum proteins was performed by an agarose gel technique that detects strong and weak protein bands in the gamma globulin region of the electrophoretogram (1) and is more sensitive than similar zone electrophoresis tests (6). The protein bands were identified by immunofixation as previously described (5).

Immunoblotting. Immunoblotting of serum IgG antibodies specific to HIV was performed by a procedure used to identify virus-specific oligoclonal IgG in cerebrospinal fluid (7), modified as follows. The active HIV-infected cell culture preparation, banded in sucrose for concentration and purification, was obtained from Electro-Nucleonics, Inc., Silver Spring, MD 20904.

The HIV was inactivated in a 1 g/L solution of Triton X-100 surfactant before use. Nitrocellulose strips were incubated overnight at room temperature, with a 10-fold concentrated HIV preparation derived from an H-9 cell line infected with HIV.

The entire procedure was performed under the hood. In the morning, the strips were briefly washed in phosphate-buffered saline and incubated at room temperature for 1 h in Tris-buffered saline, pH 7.4, containing, per liter, 60 g of bovine serum albumin and 5 g of Tween 20. Electrophoresis was performed in serum samples diluted 100- to 400-fold. Immediately after the end of the electrophoresis, the strips with the adsorbed HIV antigens were washed in phosphate-buffered saline and laid on top of the gels. Several layers of filter paper, a glass sheet, and a 0.9-kg weight were added. Passive transfer of proteins to nitrocellulose strips was allowed for 40 min. The strips were briefly washed in phosphate-buffered saline and their nonspecific binding sites were blocked with a 200 g/L solution of normal goat serum in phosphate-buffered saline for 30 min. After a brief wash, the strips were incubated at 37 °C for 2 h with rabbit anti-human IgG antiserum diluted 200-fold in blocking solution. The strips then were washed for 15 min in a 0.5 g/L solution of Tween 20 detergent in phosphate-buffered saline, with three changes of the solution. The procedure was completed by using the peroxidase "ABC" kit (Vector Lab., Burlingame, CA 94010) and development with 4-chloro-1-napththol. To prove the specificity of the method, we included seronegative samples from a normal person and from a patient with monoclonal gammopathy. Seropositive samples were blotted to nitrocellulose precoated with the supernatant fluid from a non-infected H-9 cell line and with human T-cell lymphotropic virus type 1 (HTLV-1).

Results

Figure 1 shows serum protein electrophoretic patterns of HIV-antibody-negative samples and their corresponding immunoblots. The A pattern is a typical normal control obtained by the agarose gel electrophoretic technique showing a diffuse γ-globulin zone. The C pattern, from a patient with monoclonal gammopathy, shows a strong monoclonal band in the γ-globulin zone identified as IgG κ. Their corresponding immunoblots, B and D, show no reactivity with the HIV antigens.

In Figure 2, A is the serum protein electrophoretic pattern of an HIV-antibody-positive sample with diffuse hypergammaglobulinemia. B is the immunoblot of the same sample showing a positive reaction with the HIV antigens in the γ-globulin zone. C is the serum protein electrophoretic pattern of an HIV-antibody-positive sample showing OIB in the γ-
globulin zone identified as IgG κ. D is the immunoblot of the same sample; it shows that both the OIB and the diffuse γ-globulin reacted strongly with the HIV antigens. E is an immunoblot control of sample C, showing no reaction when the nitrocellulose paper was precoated with non-infected H-9 cell culture supernate. Also, no reaction was evident with sample C when the nitrocellulose paper was precoated with an HTLV-1 preparation.

Discussion

Electrophoretic analysis of serum samples from HIV-infected persons demonstrated discrete OIB within an intense γ-globulin zone. It indicates two simultaneous processes: (a) a polyclonal B-cell hyperactivation and (b) a selective B-cell oligoclonal proliferation to secrete uniform IgG bands. In these studies we presented evidence of both of these processes by the positive reactions between the HIV-antibody-seropositive samples and the viral antigens. While diffuse hypergammaglobulinemia is a general characteristic of viral infections, the OIB indicate a modified cellular and humoral immune response of the host to the HIV infection.

It is estimated at this time that between one and two million persons have been infected with HIV (8). The central question is: what is the clinical course of the HIV-antibody carriers? Biochemical markers are needed to study the course of HIV infection, the effects of therapy, and to assess clinical prognosis. The direct association of OIB with HIV antigens suggests the use of OIB as markers to follow the course of HIV infection.

Because the interval between initial HIV infection and development of AIDS is lengthy, we are continuing periodic testing of asymptomatic HIV-antibody carriers, to determine if appearance, persistence, or disappearance of OIB may predict the clinical course of HIV infection.

In some cases, instead of strong OIB originally present in the serum protein electrophoretic patterns, weak bands have been observed on the HIV-precoated nitrocellulose blot. In one case, an extra band appeared on the blot that was not present in the original serum protein electrophoretic patterns. This is due to variable quantitative distribution of the HIV antibodies present in the γ-globulin zone and their diverse specificity against the HIV antigens used. The use of individual antigens such as p24, against HIV-antibody-positive serum samples with OIB, may provide additional information of the specificity of OIB.

References


Interference of Fetal Hemoglobin with the Spectrophotometric Measurement of Carboxyhemoglobin

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We measured the concentration of carboxyhemoglobin (HbCO) in blood samples from 32 neonates by spectrophotometry (IL282 CO-Oximeter) and gas chromatography, finding a strong positive correlation (r = 0.89) between the concentration of fetal hemoglobin (Hb F) and HbCO as measured by spectrophotometry, but not by gas chromatography. Thus, Hb F interferes with the determination of HbCO by spectrophotometric techniques by falsely increasing apparent HbCO in direct proportion to Hb F. We conclude that, when Hb F is known or suspected to be present, blood HbCO cannot be reliably determined by methods based on spectrophotometry.

Additional Keyphrases: neonates · pediatric chemistry · assessing hemolysis rate in newborns

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1 Nonstandard abbreviations: Hb, total hemoglobin; Hb F, fetal hemoglobin; HbCO, carboxyhemoglobin; TcSO2, transcutaneous oxygen saturation.