SEQUENTIAL CHANGES IN ANTIBODY LEVELS TO THE ENV AND GAG ANTIGENS IN HUMAN IMMUNODEFICIENCY VIRUS INFECTED SUBJECTS

N. MANCA ⊕ *, F. di MARZO VERONESE ⊙, D.D. HO ⊗ ⋆, R.C. GALLO ⊕, M.G. SARNGADHARAN ⊙

○ Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD. 20205.
⊗ Department of Cell Biology, Bionetics Research, Inc., Rockville, MD 20850.
⋆☆ The Infectious Disease Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114.

Key words: HIV - Antibody - Env antigens - Gag antigens.

Sera from 51 HTLV-III (human immunodeficiency virus, HIV)-antibody positive subjects consisting of 21 asymptomatic individuals and 15 ARC and 15 AIDS patients were analyzed for their serological profiles toward the viral antigens. One of the asymptomatic subjects only showed a p24 reactivity in the immunoblot, but antibodies to the env antigens were clearly identified by immunoprecipitation of viral antigens (RIP) followed by SDS-polyacrylamide gel electrophoresis. RIP patterns of different subjects and even different bleeds from the same subjects showed a varying reactivity to the gag antigens whereas the reactivity towards the env antigens appeared to be generally stable. RIP analysis of sequential sera of virus-infected individuals indicated a pattern consistent with an initial steady rise of antibody reactivities to the gag antigens relative to the reactivities to the envelope antigens. These reactivities reached a plateau and then slowly declined. While all sera tested had antibodies to the envelope antigens gp160, gp120 and gp41, 86% of the asymptomatic subjects, 67% of the ARC patients and only 33% of the AIDS patients had antibodies to the gag proteins p24 and pr53gag.

INTRODUCTION

The primary etiologic agent of the acquired immunodeficiency syndrome (AIDS) and AIDS-associated diseases has been identified as a human T-lymphotropic virus, HIV (3, 6, 9). The most compelling evidence for the association of HIV with AIDS is the highly specific correlation between the presence of serum antibodies directed against HIV antigens and AIDS (11, 12). Nearly 100% of the AIDS and AIDS related complex (ARC) patients and up to 70% of asymptomatic homosexual men at risk for AIDS were antibody-positive (5, 1, 19). On the contrary, antibodies to HIV were absent in people with no risk for AIDS. The major HIV-gene products identified in immunoblot assays are: the env gene products, gp120 and gp41 (1, 17); the pol gene products p66, p51 and p31 (13, 15), and the gag proteins p24 and p17 (16). Antibodies to these are observed in a majority of sera from HIV-infected individuals and thus represent the char-
characteristic serological profile of HIV-infection. Not all antibody-positive sera react with all the above antigens, but most react with the envelope transmembrane protein gp41.

In the present report, we compare the immunological profiles seen in the immunoblot assay using viral extracts with those obtained in immunoprecipitation (RIP) and sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of labeled viral proteins in cell extracts. We also study the emergent antibody pattern at seroconversion and the changes in the pattern in individual subjects as a function of time, in an effort to understand the significance, if any, of these changes.

**MATERIALS AND METHODS**

**Sera Samples.** Sera were obtained from a collection at the Infectious Diseases Unit of the Massachusetts General Hospital, Boston. A total of 164 sera, consisting of 87 samples from 21 healthy homosexual males at risk for AIDS and 62 samples from 15 patients with ARC, collected at varying intervals, and 15 samples from 15 patients with AIDS, were analyzed in this study. The antibody positivity of the study subjects had already been established prior studies. Among the healthy homosexuals 3 seroconverted during the study, while the others had antibodies to HIV in the earliest samples analyzed. Also during the study 3 previously healthy individuals developed ARC and the clinical status of one of the ARC patients changed to AIDS.

**Metabolic Labeling, Radioimmunoprecipitation, and SDS-PAGE.** H9/HTLV-IIIb cells were grown in RPMI 1640 containing 100 μg/ml penicillin, 50 μg/ml streptomycin, and 2 mM L-glutamine (Gibco Laboratories, Grand Island, NY), 20% v/v heat-inactivated (56°C for 30 min.) fetal calf serum (FCS, Gibco), in a humidified incubator at 37°C in 6% CO₂. Cells were radioactively labeled by incubation for 18 hours in leucine-free medium containing [3H]-leucine (New England Nuclear) at a concentration of 100 μCi/ml (17).

Labeled H9/HTLV-IIIb cells (3 × 10⁷) were washed in phosphate buffered saline (PBS) and suspended in 5 ml of 10 mM sodium phosphate (pH 7.2) containing 0.5% NaCl, 1% Triton X-100, 0.5% sodium deoxycholate and 0.1% sodium dodecyl sulfate (PBS-TDS). The cells were disrupted at 4°C by repeated aspiration through a 22 gauge needle. The lysates were pre-absorbed for 3 hrs. at room temperature with 3 ml of a 10% suspension of protein A Sepharose-CL4B containing 80 μl of a normal human serum. They are subsequently clarified by centrifugation for one hour at 100,000 x g. Aliquots of the clarified lysate (0.5 ml) were used in immunoprecipitation by incubation for 18 hours at 4°C with 10 μl of the test human serum and 100 μl of a 10% suspension of protein A Sepharose. Immunoprecipitates were collected by centrifugation at 2,000 g for 10 minutes washed repeatedly in PBS-TDS, suspended in 50 μl of 0.65 M Tris hydrochloride (pH 6.7), 1% sodium dodecyl sulfate, 10% glycerol, 2.5% 2-mercaptoethanol and 0.1% bromophenol blue, heated for 2 minutes at 90°C and applied to 10-20% slab polyacrylamide gels for electrophoresis. The radioactive bands were identified by fluorography.

**Western Blot Immunoassay.** Detergent lysates of HIV were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins were electrophoretically transferred to nitrocellulose sheets according to the methods of Towbin et al. (14). These sheets were then incubated at 37°C in a 5% solution of nonfat dry milk containing 0.01% Antifoam (Sigma) and 0.0001% Merthiolate (blocking medium) to block nonspecific protein binding sites. Strips containing representative profiles of viral antigens were cut from these sheets and incubated overnight in individual test tubes with 2.5 ml of blocking medium containing 4% normal goat serum and 25 μl of the human test serum. The strips were washed three times with 0.5% sodium deoxycholate, 0.1 M NaCl, 0.5% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and 10 mM sodium phosphate (wash medium). The washed strips were incubated for 30 minutes in 2.5 ml of blocking medium containing 4% normal goat serum with 2.5 × 10⁶ cpm of ¹²⁵I-labeled goat IgG reactive against human Fc fragment and μ chain. The strips were washed again as before, dried, mounted, and autoradiographed.

**RESULTS**

**Seroconversion to HIV: Emergent Antibody Pattern.** Six sequential sera from a healthy individual, collected at 2-6 month intervals during 22 months, were tested by the immunoblot to look for specific antibodies to HIV antigens. The results obtained with the first 4 samples are shown in Fig. 1, Panel A. The first appearance of any antibody was in sample 3, (lane 3) but the reactivity was limited to the gag protein p24. However, the subsequent 3 bleeds (one shown in lane 4) contained antibodies to most of the HIV antigens usually seen in the immunoblots (17). This finding, initially made in our laboratory more than 2 years ago, suggested that antibodies to the gag antigen p24 might be the earliest sign of seroconversion to HIV.

There were some inherent limitations in the system to follow changes in antibody patterns...