Brief Definitive Report

Immunological Crossreactivity Between the Human Immunodeficiency Virus Type 1 Virion Infectivity Factor and a 170-kD Surface Antigen of Schistosoma mansoni

By Jamal Khalife,* Jean-Marie Grzych,* Raymond Pierce,* Jean-Claude Ameisen,* Anne-Marie Schacht,* Hélène Gras-Masse,† André Tartar,† Jean-Pierre Lecocq,§ and André Capron*

From the *Centre d'Immunologie et de Biologie Parasitaire, Unité Mixte INSERM, U167-CNRS 624, and †Unité de Chimie des Biomolécules, Institut Pasteur, 59019 Lille; and §Transgène S.A., 68000 Strasbourg France

Summary

A monoclonal antibody (mAb) directed against a synthetic peptide derived from the sequence of the human immunodeficiency virus type 1 (HIV-1) regulatory protein virion infectivity factor (vif) labeled the surface of Schistosoma mansoni schistosomula by indirect immunofluorescence. Western blotting showed that two S. mansoni proteins of 170 and 65 kD were recognized by the mAb. Sera from 20% of S. mansoni-infected HIV-seronegative individuals tested recognized the PS4 peptide in an ELISA as did sera from S. mansoni-infected rats. Sera from individuals seropositive for HIV-1, but without schistosomiasis, that reacted with the vif peptide also recognized a 170-kD S. mansoni protein. This crossreactive S. mansoni antigen appears to be a target of immunity in vivo since passive transfer of the mAb VIF-CD3 to naive rats had a protective effect against a challenge infection with S. mansoni cercariae.

One of the main characteristics of HIV infection in Africa is that it principally affects populations in areas of chronic parasitic infections. This has raised the possibility of a mutual influence on the development of the respective diseases in the case of concomitant infections. In this context, seroepidemiological studies suggested a relationship between HIV-1 and both malaria and schistosome infections (1, 2). In schistosomiasis, it was shown that patients had a high proportion of antibodies against HIV-1 and that there was no antigenic crossreactivity between Schistosoma mansoni and HIV-1 structural proteins (3). While investigating a possible crossreactivity between HIV-1 proteins and S. mansoni antigens, we confirmed the absence of crossreactivity between HIV-1 structural virion proteins (gag, pol, env) and S. mansoni. However, we demonstrate an immunological crossreactivity between an epitope of virion infectivity factor (vif) one of the six nonstructural regulatory proteins encoded by the HIV-1 genome, and an S. mansoni surface antigen.

Materials and Methods

Parasites and Antigen Preparations. A Puerto Rican strain of S. mansoni was maintained in Biomphalaria glabrata snails and golden hamsters. Schistosomula were prepared by skin penetration (4). Adult worms were prepared by hepato-portal perfusion of infected hamsters. Soluble extracts of adult worms and cercariae were produced as previously described (5). The complete coding sequence of the LAV-1 Bru isolate of the VIF protein was expressed in Escherichia coli, as described elsewhere (r vif) (6), and purified to >60% for use in Western blotting analysis.

Sera. LOU rats, percutaneously infected by 1,500 cercariae, were the source of infected rat sera. Human infection sera were obtained from children living in an endemic area in Kenya that has been extensively reported (5, 7–8). These sera were seronegative for HIV-1 structural proteins (ELAVIA; Pasteur Diagnostic, Lille). HIV-1 sera were obtained from European individuals seropositive for structural proteins gag, pol, and env who had never been in contact with S. mansoni. Normal human sera were obtained from individuals negative for both infections.

Synthetic Peptides. The vif peptide comprising amino acids 155-168 was selected as a probable epitope after algorithm analysis for criteria of hydrophilicity, helicity, mobility, and amphiphilicity, and was synthesized by solid-phase methodology (9, 10). The amino acid sequence of this peptide, named PS4, was TPKKIKPPLPSVTK (11). The composition of the peptide was confirmed by amino acid analysis, and the degree of purity was assessed by C-18 reversed-phase HPLC. Two other vif peptides were used throughout the experiments: the vif 32-50 peptide named PS2 was SGKARGWFVRHHVESPHPR; the vif 85-105 peptide named PS3 was VSI6WRKKRSTQVDPFELAD. A control peptide, PEI (847-861), derived from the sequence of the HIV env protein (IPR-RJBGLERILL), was used as a control.

Rat mAbs. mAbs to the vif peptide were obtained in a homol-
ogous hybridization system (12). Male LOU rats were injected intraperitoneally with 100 µg of purified vif peptide (PS4) conjugated to tetanus toxoid in the presence of CFA followed by two injections of 100 µg of peptide alone in IFA. One clone (VIF-CD3) of the IgM isotype was selected for further investigations and used for the production of ascitic fluids.

Ascitic fluids (1 ml) were injected intravenously into five LOU rats in each group (10 wk old), which had been infected with 1,200 S. mansoni cercariae 4 h previously. Parasite burdens were evaluated 3 wk later, as described previously (13). Results were compared by student's t test.

**Western Blotting and Indirect Immunofluorescence.** Electrophoresis and Western blotting of proteins were as previously described (14). mAbs and human sera were used at a 1:100 dilution, and bound antibody was detected with peroxidase-labeled mouse anti-rat IgM (Serotec) or rabbit anti-human Ig (H+L; Institut Pasteur Production). Surface binding of mAB antibodies was detected by indirect fluorescence on live or fixed schistosomula prepared as described elsewhere (15).

**ELISA and Inhibition Assay.** Microtiter plates (Nunc, Roskilde, Denmark) were coated with 100 µl of adult worm antigens at 10 µg/ml or peptides at 5 mg/ml. For inhibition experiments, the mAb anti-vif peptide was preincubated for 3 h at 37°C with peptides at different concentrations. In all experiments, the antibodies were incubated overnight at 4°C, and the wells were then washed with PBS/0.4% Tween. The anti-Igs used were the same as described above. Each test was assessed in duplicate. Results are expressed as mean ± SD.

**Results and Discussion**

Of the 550 hybrid cell supernatants screened using the ELISA, four showed specific anti-vif peptide (PS4) reactivity. These antibodies were tested for their ability to react with the E. coli–expressed recombinant vif protein (Fig. 1 A), and one clone named vif-CD3 (IgM isotype) was retained for further investigations. Ascitic fluids were produced and used to probe Western blots of whole S. mansoni cercarial antigen, revealing two bands at 170 and 65 kD (Fig. 1 B). The 170-
kD band was also detected in adult worm products, along with a weak band at 43 kD (data not shown). The mAb vif-CD3 in ascitic fluids bound to the surface of live or fixed schistosomula up to a dilution of 1:800 (Fig. 2). A kinetic study showed that only 10% of 3-h skin schistosomula gave a weak membrane fluorescence, whereas all schistosomula gave a strong surface fluorescence after an 18-h incubation in MEM. These results suggested that the expression of this epitope at the surface may be developmentally regulated. Negative controls used included an unrelated IgM class rat mAb (directed against an *Onchocerca volvulus* antigen) that gave no fluorescence. An ELISA was used to further assess the specificity of binding of mAb VIF-CD3 to *S. mansoni* antigens. The PS4 peptide inhibited (up to 60%) the binding of the mAb to soluble adult worm antigens, whereas another peptide derived from the vif sequence (PS3) showed no inhibition. When the mAb was preincubated with intact schistosomula and then tested for its ability to bind to peptide PS4, a significant (45%) inhibition was obtained; confirming that the crossreactive antigens were located at the surface of the larvae.

The possibility that the crossreactive epitope expressed at the surface of schistosomula might be a target of immunity was addressed by the passive transfer of 1 ml of mAb VIF-CD3 to rats 4 h before infection with 1,200 cercariae. The parasite burden was evaluated 3 wk later by liver perfusion was addressed by the passive transfer of 1 ml of mAb VIF-CD3 to rats 4 h before infection with 1,200 cercariae. The parasite burden was evaluated 3 wk later by liver perfusion

...
References


