

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

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### **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 TPKK-*IKPPLPSVTK* (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 *VSIEWRKKRYSTQVDPELAD*. A control peptide, PE1 (847-861), derived from the sequence of the HIV env protein (*IPR-RIRBGLERILL*), was used as a control.

**Rat mAbs.** mAbs to the vif peptide were obtained in a homol-

ogenous hybridization system (12). Male LOU rats were injected intraperitoneally with 100  $\mu$ g of purified vif peptide (PS4) conjugated to tetanus toxoid in the presence of CFA followed by two injections of 100  $\mu$ g 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.

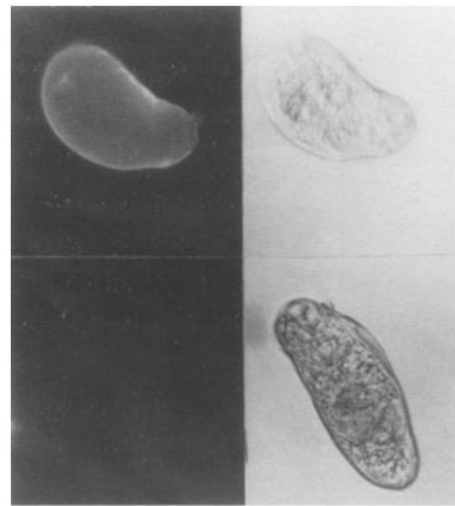
Ascitic fluids (1 ml) were injected intravenously into five LOU rats in each group (10 wk old), which had been infected with 1,200 *S. mansoni* cercariae 4 h previously. Parasite burdens were evaluated 3 wk later, as described previously (13). Results were compared by student's *t* test.

**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  $\mu$ l of adult worm antigens at 10  $\mu$ g/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  $\pm$  SD.

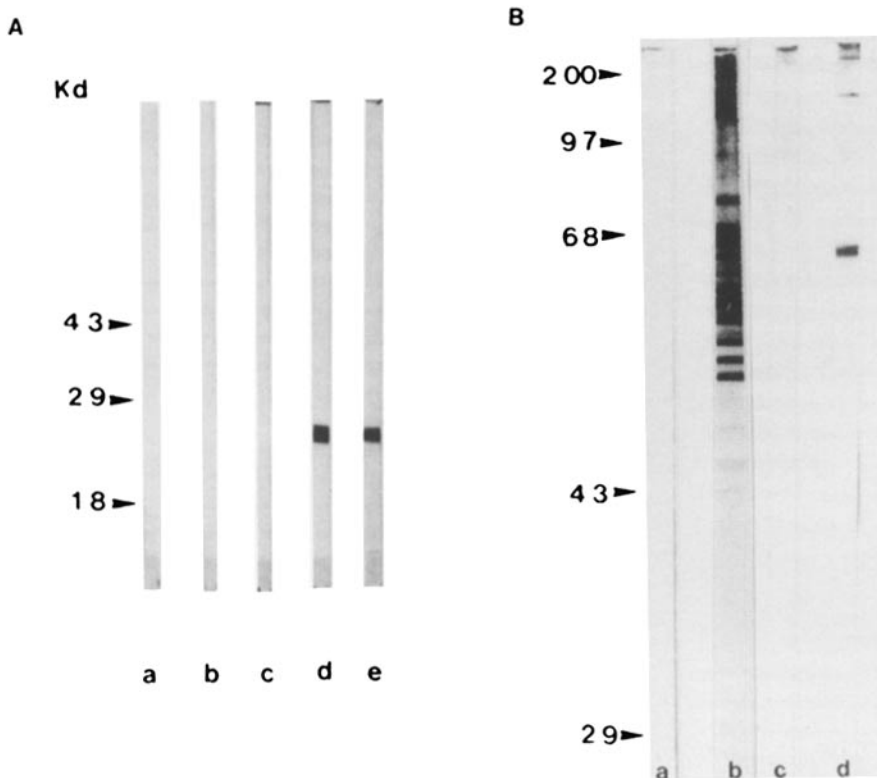
## Results and Discussion

Of the 550 hybrid cell supernatants screened using the ELISA, four showed specific anti-vif peptide (PS4) reactivity.



**Figure 2.** Localization of the antigenic sites recognized by the mAb VIF-CD3 directed against PS4 vif peptide. Indirect fluorescence was performed on fixed *S. mansoni* schistosomula incubated with the mAb and developed with FITC anti-rat (H+L). (top) mAb vif-CD3; (bottom) control mAb IgM anti-*O. volvulus*.

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-



**Figure 1.** Epitopic identity between the HIV-1 vif protein and *S. mansoni* antigens. (A) Immunoblot of vif protein (purified up to 60%) with normal rat serum (NRS) (a); sera from rats immunized with tetanus toxoid (b); rat mAb IgM anti-*Onchocerca volvulus* (unrelated IgM) (c); rat IgM mAb anti-vif peptide PS4 (VIF-CD3) (d); serum from a rat immunized with the PS4 peptide (e). (B) Identification of *S. mansoni* components recognized by the mAb VIF-CD3. Western blot was performed on cercarial antigens extracted in TNSTE. (Lanes a-d) Normal rat serum; sera from rats infected 8 wk previously with *S. mansoni*; unrelated rat mAb IgM; and the IgM mAb VIF-CD3 anti-PS4, respectively.

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 and showed a significant level of protection ( $p < 0.02$ ) compared with control groups injected with saline in four separate experiments (46, 40, 34, and 49%). In comparison, the protection level that can be conferred by an anti-*S. mansoni* mAb is ~60% (12). Passive transfer of an unrelated mAb of the same isotype (IgM) did not confer any protection (two of the four experiments). These results suggest that the *S. mansoni* epitope defined by mAb VIF-CD3 represents a potential target of effector mechanisms operating in vivo.

To determine the relevance of these observations to schistosomiasis, we investigated whether crossreactive antibodies were produced during the course of human and rat infections. A total of 61 sera from *S. mansoni*-infected individuals that were all negative for HIV-1 structural proteins were tested for their capacity to bind to the PS4 peptide in ELISA. 12 reacted significantly (data not shown), whereas no sera gave

a positive result with the PS2 vif peptide nor with the control env peptide (PE1). None of the 18 sera from HIV-1-negative and *S. mansoni*-negative Europeans recognized any of the peptides. To rule out the possibility that latent HIV-1 infection (16) might be involved at the presence of anti-PS4 antibodies in the sera of the *S. mansoni*-infected patients, sera from experimentally infected rats were also tested in the PS4 ELISA. Sera from three of six 6–8-wk infected rats reacted significantly with PS4 when compared with normal rat sera, but did not recognize the control peptides. Conversely, sera from 11 European individuals seropositive for HIV-1 and with no schistosomiasis were tested for the presence of antibodies to both the PS4 vif peptide and to *S. mansoni* cercarial antigens. 5 of 11 gave a positive PS4 ELISA, three others were borderline. Of the positive or borderline cases, six recognized the 170-kD *S. mansoni* antigen, and one of them recognized an additional 62-kD antigen. Two of eight that recognized PS4 did not recognize any specific *S. mansoni* band, suggesting that the PS4 peptide might not represent the complete crossreactive epitope, or alternatively, that only a part of PS4 is the crossreactive epitope.

It appears from these results that a proportion of individuals infected either with HIV-1 or *S. mansoni* produce antibodies to a common epitope that is at least partly defined by the PS4 peptide and correspond to a *S. mansoni* 170-kD surface antigen that appears to be a potential target of protective immunity. For this reason, we have purified the crossreactive antigen fraction in order to undertake its molecular cloning and to define more exactly its relationship to the HIV-1 vif protein. No hybridization was obtained between vif cDNA and *S. mansoni* genomic DNA, suggesting that the crossreactivity may involve a very limited DNA sequence only; or amino acid peptide sequence homology that does not correspond to DNA sequence, or alternatively, to a conformational epitope. Our results raise the question of the possible influence of a prior humoral and cellular response to a crossreacting *S. mansoni* antigen on an HIV infection. We are at present testing the possibility that anti-*S. mansoni* immune mechanisms may exert an effect on HIV-1 replication or HIV-1-infected cells.

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