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INTRACELLULAR LOCALIZATION OF RECOMBINANT VACCINA VIRUS PRODUCED HIV ANTIGENS AND THEIR USE FOR CONFIRMATION OF HIV SEROPOSITIVITY

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Summary. — Recombinant vaccinia strains vC5 and vE234L expressing the rp50 and rgp160/rgp120 recombinant proteins were used in immunoblot and immunofluorescence assays. No false reactions were found, although 30 sera giving false reactivity with *gag* or *env* encoded proteins in a commercial immunoblot assay were included into the test panel. We recommend the recombinant protein-based assay for confirmation and discrimination of HIV seropositivity.

Key words: *HIV seropositivity*; *recombinant vaccinia strains*, *false positive results*; *recombinant proteins*

With the beginning of mass blood screening for HIV seropositivity the urgent need for a reliable confirmation test has become evident. Although the immunoblot (IB) with natural virus antigens is very informative and widely used, it may occasionally give false positive results which are very difficult to differentiate from true positives (Tersmette *et al.*, 1988; Bukrinsky *et al.*, 1988). These false positive reactions are usually caused by HLA proteins present in viral lysate (Henderson *et al.*, 1987) or by cross reactions of partially denatured HIV antigens with HIV-unrelated antibodies (Resnick and Shapshak, 1987).

To eliminate the false reactions we have used recombinant vaccinia-produced antigens instead of the natural ones. As previously shown (Bukrinski *et al.*, 1989) the use of recombinant protein rp50 endoced by HIV *gag* and carried by vaccinia strain vC5 allows to discriminate between real and false reactions with *gag*-endoced proteins. In this paper we further characterize the recombinant proteins and demonstrate that IB with recombinant *gag* (produced by vC5) and *env* (produced by vE234L) antigens gives no false reactions retaining diagnostic properties of natural antigen-based IB. The tecombinant vaccinia strains may be also used for preparation of cells for indirect immunofluorescence assay.

Construction of vC5 was previously described (Bukrinsky *et al.*, 1989). To construct the vE234L *Eco*RI-*Xho*I fragment containing the whole *env* gene of the BH10 strain (Ratner *et al.*, 1985) was digested with *Bal*31 exonuclease

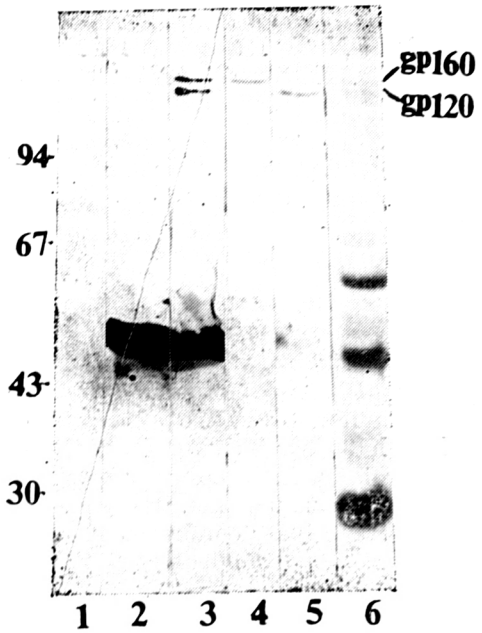

Fig. 1

Characterization of recombinant vaccinia-produced antigens by IB assay
CV1 cells infected with vSC8 (control strain, lane 1), vC5 (HIV *gag* carrying strain, lane 2), and vE234L (HIV *env* carrying strain, lanes 4, 5) at multiplicity of 30 PFU/cell (lanes 2, 4) or 2 PFU/cell (lane 5) were lysed after 6 hr (lanes 2, 4) or 24 hr (lane 5) and fractionated in 10% SDS-PAGE, HIV-specific proteins were revealed by incubation with serum of infected patient. Mixture of vC5 and vE234L infected cells was fractionated in lane 3. This lane represents IB strips used as recombinant IB assay. H9 cells infected with HTLV-IIIB HIV strain were fractionated in lane 6. Molecular masses of protein standards are shown at the left.

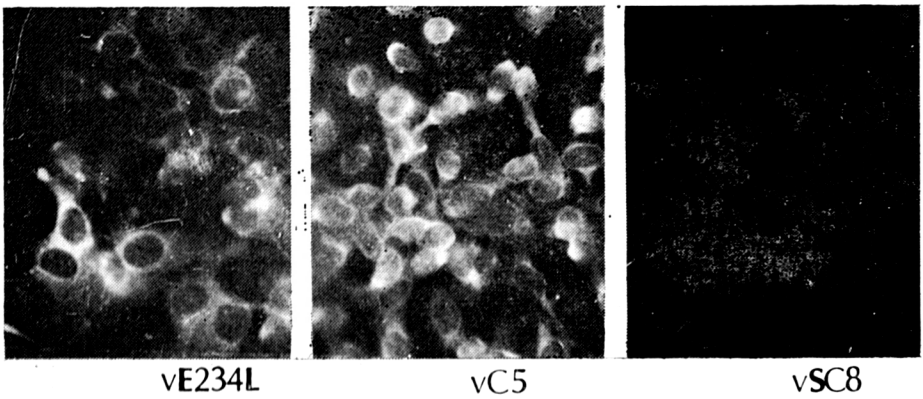

Fig. 2

Detection of recombinant antigens in CV-1 cells by indirect immunofluorescence
Subconfluent monolayer of CV-1 cells infected with 5 PFU/cell of one of vaccinia strains for 24 hr was fixed with 96% ethanol and incubated with 1 : 20 dilution of AIDS patient serum for 1 hr, 37 °C. Reaction was detected by incubation with fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG and examined by fluorescence microscopy.

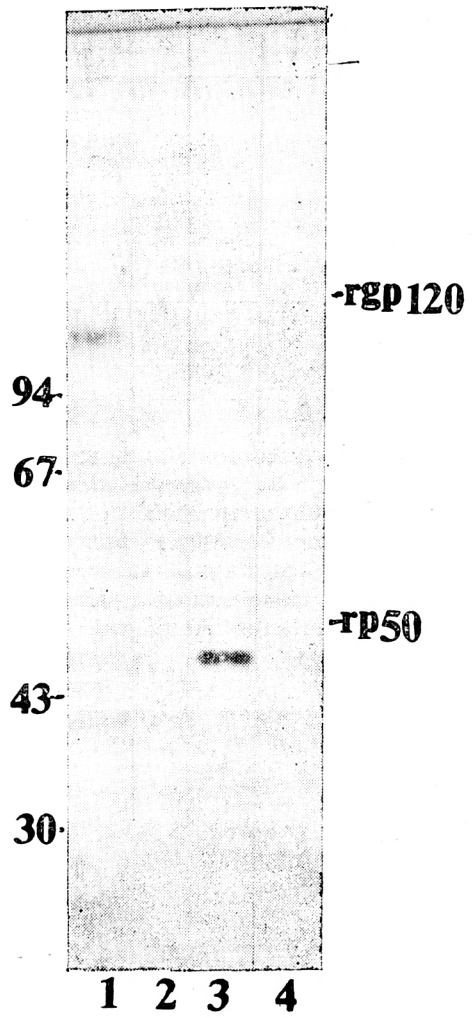

Fig. 3
Intracellular localization of recombinant antigens
CV-1 cells infected with vC5 (lanes 3, 4) or vE234L (1, 2) were fractionated as described in the text and proteins were revealed by IB assay with AIDS patient serum. Lanes 1, 3 — membrane fraction, 2, 4 — cytosol fraction.

from the *Eco*RI site. The resulting fragment carrying the *env* gene with a 110 bp leader (devoid of initiation codons other than *env* gene's own ATG) was cloned into the *Sma*I site of the pSC11 (Chakrabarti *et al.*, 1985) and used for vE234L construction by recombination with WR strain of vaccinia virus. CV-1 cells infected with vE234L produced HIV-specific proteins with molecular masses of 160kD and 120kD, specifically recognized by sera of HIV-infected patients (Fig. 1). These proteins had the same mobility in SDS-PAGE as native HIV-specific glycoproteins gp160 and gp120. Processing of the 160kD protein in CV-1 cells was rather efficient and after 24 hr we could not detect this protein by IB assay (Fig. 1, lane 5). We

Table 1. Comparative study of immunoblot assays based on natural and recombinant HIV antigens

		Positive sera	Negative sera	
		100 samples	False positive 30 samples	Negative 100 samples
Natural IB (DuPont)	Reactive	100	30	0
	Nonreactive	0	0	100
Recombinant IB	Reactive	100	0	0
	Nonreactive	0	30	100

designated the recombinant proteins produced by vE234L as rgp160 and rgp120.

For further characterization of recombinant proteins we studied them by indirect immunofluorescence assay using pooled sera of HIV-infected persons. Both vC5 and vE234L infected cells showed positive fluorescence in the perinuclear zone indicating that recombinant HIV-specific proteins were bound to cell membranes (Fig. 2). This was confirmed by cell fractionation

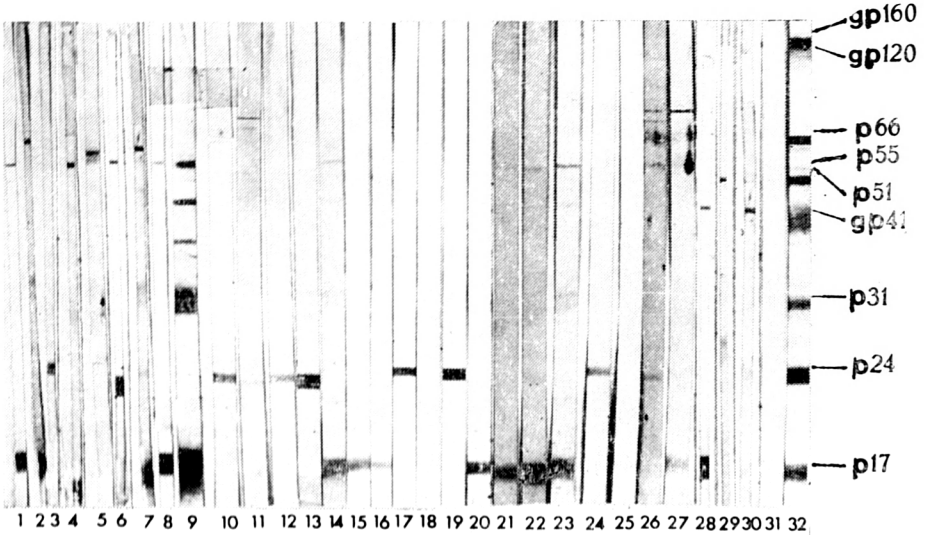

Fig. 4

False-positive reactions detected by commercial (DuPont) IB assay
1–30 — serum samples from healthy uninfected subjects demonstrating false reactivity; 31 — DuPont negative control sample; 32 — DuPont positive control sample.

and subsequent IB analysis. CV-1 cells infected with recombinant vaccinia strains were suspended in the buffer containing 10 mmol/l Tris-HCl, pH 7.4, 10 mmol/l NaCl, 30 mmol/l $MgCl_2$, and 5 mmol/l PMSF and broken by homogenization. The nuclei were removed by centrifugation and the cytoplasmic fraction was overlaid onto 41% sucrose prepared on the same buffer and centrifuged for 2 hr at 21 000 rev/min in SW27.1 bucket rotor. Proteins from the cytosol (upper layer) and membrane (found between cytosol and sucrose) fractions were precipitated by acetone and analysed by IB. One can see from Fig. 3 that membrane fraction was heavily enriched in recombinant proteins. As far as the rp50 gene includes the intact HIV p17 coding sequence (Bukrinsky *et al.*, 1989) we believe that rp50 is myristoilated at its NH_2 terminus, as it was described for p17 (Mervis *et al.*, 1988). This anchors rp50 to the membrane. The rp50′ natural counterpart —HIV p55 — is also known to be membrane-bound and such localization favours the correct processing of this protein.

The vC5 and vE234L infected cells were lysed and used for production of IB strips. The sensitivity of this IB assay was compared to DuPont IB by titrating positive control serum. Up to 10^{-4} dilution, the serum could be detected by our IB, while DuPont test was about 5 times more sensitive. We tested a panel of pooled sera using recombinant and commercial IB assays. This panel included confirmed seropositive and negative samples, as well as 30 false positive sera (Table 1). These false positive sera were collected from healthy persons without known risk factors. The reaction pattern in DuPont IB was unchanged for over 6 months.

Results of testing positive and negative samples in recombinant and natural IB assays were quite similar. Dramatic differences were found when false positive samples were tested. As previously shown the majority of false reaction in IB was directed against the *gag*-encoded proteins (Resnick and Shapshak, 1987). However, sometimes such reactions occur against proteins comigrating with *env*-encoded products (Bukrinsky *et al.*, 1988). The most common type of false reactions seen in this study was anti-p17 and anti-p24. Some samples demonstrated reactivity to p66 (samples 2, 7, 19, 26, 27 on Fig. 4), gp41 (9, 14, 23), p31 (9, 13, 23). The majority of samples reacted with a protein of M_r 51/55 kD.

None of these sera interacted with the recombinant proteins. They were also negative in the Abbot recombinant ELISA confirming the false nature of reactivity in IB. Some of these sera were tested by immunofluorescence assay using either H9 cells infected with HTLV-IIIB strain or CV-1 cells infected with vC5 or vE234L. These assays gave similar results (not shown) also demonstrating the negativity of the samples.

Our data indicate that recombinant HIV proteins obtained in heterologous systems may help discriminate between real and false positive results. We think that introduction of recombinant antigen-based IB will be a step towards a reliable confirmation test.

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Erratum: The editors deeply regret the mistakes which occured in vol. 34 of Acta virologica. In No. 1, Fig. 4 on page 47 was interchanged with Fig. 2 on page 46. In No. 3, Fig. 4 on page 259 was interchanged with Fig. 3 on page 258. We apologise to the authors as well as to our readers.