SHORT COMMUNICATION

An inhibition enzyme immunoassay using a human monoclonal antibody (K14) reactive with gp41 of HIV-1 for the serology of HIV-1 infections

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An inhibition enzyme immunoassay (IEIA), using a human monoclonal antibody (K14) reactive with gp41 of HIV-1, was evaluated for its applicability to the serology of HIV-1 infections. Using panels of serum samples from seronegative and confirmed HIV-1-seropositive individuals, it was shown that all the HIV-1-positive samples in a panel from The Netherlands and 97% of the HIV-1-positive samples from Tanzania were identified by this IEIA. Six per cent of the IEIA-positive samples from Tanzania could not be confirmed in other assays. Testing of serial dilutions of serum samples from African individuals with confirmed HIV-1, HIV-2 or HIV_{ANT70} infections in the K14 IEIA, indicated that a HIV-1-specific assay based on this principle may be developed.

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Introduction

Several enzyme immunoassays (EIA) based on different techniques and principles have been developed for HIV serology [1]. Most of the EIA for detection of HIV-1 antibodies give positive reactions with 60–90% of the HIV-2-antibody-positive serum samples [2]. Combination assays have been developed [3], which detect both HIV-1- and HIV-2-reactive sera. Since antigenic differences between HIV-1 and HIV-2 are most pronounced within their envelope glycoproteins, serological assays which discriminate between these infections are generally based on differences in reactivities of antibodies with the glycoproteins of these viruses [2–8]. These assays use synthetic peptides representing an immunodominant and partially conserved domain of gp41 of either of the viruses [5–8]. The use of inhibition EIA with monoclonal antibodies specific for such domains, may also be considered for this purpose [9].

Recently, we described a human monoclonal antibody (K14) which reacted with a conserved and immunodominant epitope of gp41 of HIV-1, but not with HIV-2 [10]. In this study we have tested large panels of human serum samples from different origins in the K14 inhibition EIA (IEIA).

Materials and methods

Serum samples

Four panels of serum samples were collected for the evaluation of the K14 IEIA. The first panel was collected from 219 HIV-1 antibody-positive asymptomatic and symptomatic individuals, and from 140 HIV-1 antibody-negative individuals in The Netherlands. The second was collected from 71 individuals in The Netherlands before and after HIV-1 seroconversion [11]. The third, (267 samples), was collected in Tanzan...
nia from individuals suspected of having AIDS or with AIDS-related symptoms. These three panels had been tested by at least one commercially available HIV-1 antibody EIA (recombinant HIV-1 EIA, Abbott Laboratories, North Chicago, Illinois, USA; Vironostika anti-HIV-1 micro-EIA system, Vironostika Teknika BV, Boxtel, The Netherlands). All positive reactions had been confirmed by Western blot analysis (WB).

The fourth panel, collected in West and West Central Africa (Ivory Coast, Cameroon and Gabon), consisted of seven positive samples (WB) for HIV-2 and HIV-1, 35 HIV-2 WB-positive samples with a negative or indeterminate HIV-1 WB reactivity, nine HIV-1 WB-positive samples with an indeterminate HIV-2 WB reactivity and two samples from individuals infected with a recently described HIV variant, HIV_{ANT70} [12]. These samples were tested in the HIV-2 WB NEW LAV BLOT II (Pasteur, 's-Hertogenbosch, The Netherlands) and either in the Du Pont HIV-1 WB (Du Pont, 's-Hertogenbosch, The Netherlands) or the Organon HIV-1 WB (Organon Teknika). Samples, reactive with HIV-2 WB and HIV-1 WB were also tested in the INNO-LIA HIV-1/HIV-2 Ab (Innogenetics, Antwerp, Belgium). Samples reactive with two envelope glycoproteins in the HIV-2 WB or HIV-1 WB were considered HIV-2 or HIV-1 antibody-positive, respectively. All other WB profiles were considered indeterminate.

**K14 IEIA**

Protein-A chromatography purified K14 and was conjugated to horse radish peroxidase (HRP; Boehringer Mannheim GmbH, Mannheim, Germany) using the heterobi-functional reagent n-succinimidyl-3-(2-pyridyl-dithio)-propionate (SPDP; Pharmacia, Uppsala, Sweden). The inhibition, enzyme-linked immunosorbent assay (ELISA) was performed on HIV-1-coated micro-ELISA plates (Vironostika, Organon Teknika). Twenty-five microliters of the serially diluted samples were simultaneously incubated with 75 μL of K14 conjugate 1:4000 diluted in PBS with 0.05% Tween 80, 1% Triton X100 and 5% normal goat serum. After 1 h incubation at 37°C, the plates were washed three times with PBS-Tween 80 and developed with TMB/DMSO as described [10].

Samples collected in West and West Central Africa were also tested in a K14 IEIA using micro-ELISA plates coated with gp160 (baculovirus recombinant gp160, Organon Teknika). A sample was considered positive if the inhibition percentage was ≥50% [10].

All 219 samples from symptomatic and asymptomatic confirmed HIV-1-seropositive individuals showed inhibitions of more than 70% (percentage inhibition \( \times \pm \text{s.d.} = 91 \pm 3 \)), whereas all the samples from the 140 seronegative individuals showed inhibitions of less than 30% (percentage inhibition \( \times \pm \text{s.d.} = 4 \pm 12 \)). Testing of sera from 71 individuals, which had been collected just before and within 3 months of WB-confirmed seroconversion [11], demonstrated that all the WB-confirmed positive samples showed inhibitions of more than 50% (percentage inhibition \( \times \pm \text{s.d.} = 90 \pm 9 \)), whereas all the samples collected 3 months earlier showed inhibitions of less than 30% (percentage inhibition \( \times \pm \text{s.d.} = 8 \pm 10 \); Fig. 1b).

To further evaluate the specificity of the K14 IEIA, the 267 samples from Tanzania (East Africa) were tested. Of the 110 samples from confirmed HIV-1-seropositive individuals, 102 showed inhibitions of more than 70%, five between 50 and 70% and three between 35 and 40% (percentage inhibition \( \times \pm \text{s.d.} = 91 \pm 12 \)). Of the 157 HIV-1-negative samples from this area, 131 showed inhibitions of less than 30%, 17 between 30 and 50%, six between 50 and 70% and three of more than 70% (percentage inhibition \( \times \pm \text{s.d.} = 5 \pm 24 \); Fig. 1c). The failure to detect 3% of the positive samples, may be a reflection of genetic variability of African HIV-1 strains causing certain strains not to express the K14 epitope [13,14] or of the inability of certain individuals to raise antibodies to the K14 epitope. The false-positive reactions, which are not uncommon when African sera are tested for the presence of antiviral antibodies [15,16], may be attributed in part to the presence of distinct but HIV-1-related lentiviruses not previously demonstrated in man in this region.

Analysis of 51 samples from West and West Central Africa, selected on the basis of their reaction patterns in WB against HIV-1 and HIV-2 and two samples from individuals infected with HIV_{ANT70} demonstrated that the K14 IEIA may also detect antibodies to HIV-2 and HIV_{ANT70}. Since K14 does not react in EIA with HIV-2 or HIV_{ANT70} (data not shown), the inhibition of K14 by samples from HIV-2 or HIV_{ANT70} infected individuals may be explained by steric hindrance caused by antibodies reactive with epitopes in the vicinity of the K14 epitope. As shown in Fig. 1d, all the HIV-1 WB-positive (HIV-2 WB-negative) samples showed inhibitions of more than 70% (percentage inhibition \( \times \pm \text{s.d.} = 92 \pm 6 \)). Six of the seven HIV-1 and HIV-2 WB-positive samples also showed inhibitions of more than 70% (percentage inhibition \( \times \pm \text{s.d.} = 86 \pm 6 \)). The remaining sample showed an inhibition of 62%. The 35 HIV-2 WB-positive (HIV-1 WB-negative) samples showed inhibitions between six and 85% (percentage inhibition \( \times \pm \text{s.d.} = 46 \pm 23 \)). The two HIV_{ANT70} positive samples showed 51 and 84% inhibition, respectively.

To further assess the discriminative capacity of the K14 IEIA, the 53 West and West Central African samples

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**Results and discussion**

Results of the analysis of 359 samples from The Netherlands, in the K14 IEIA are shown in Fig. 1a.
were diluted 1:10 or 1:30 before testing. As shown in Figs 1e and f, eight of the nine HIV-1 WB-positive samples remained clearly positive (>70% inhibition), whereas the inhibition of all the 35 HIV-2 WB-positive (HIV-1 WB-negative) samples, the two HIV\_\text{ANT70-positive} samples and five of the seven HIV-1 and HIV-2 WB-double-positive samples, decreased to less than 35, 35 and 45%, respectively.

As shown in Figs 1g, h and i, virtually all the inhibition values above 20% decreased about 15% when recombinant HIV-1 gp160 was used as antigen. At a 1:30 dilution the inhibition of all 35 HIV-2 WB-positive samples, the two HIV\_\text{ANT70-positive} samples and six of the seven HIV-1 and HIV-2 WB-double-positive samples decreased to less than 20, 15 and 40%, respectively, whereas eight of the nine HIV-1 WB-positive (HIV-2 WB-negative) samples remained positive (>50% inhibition).

The positive reaction with gp160-coated plates obtained with one HIV-1 and HIV-2 WB-double-positive sample at a 1:30 dilution may be explained by a HIV-1 and HIV-2 double infection. This was confirmed by a serological assay for the discrimination between HIV-1 and HIV-2 infection (INNO-LIA).

Taken together, the results indicate that the K14 IEIA is as sensitive as other commercial EIA, for the serology of HIV-1 infection in Europe. It should be realized however, that a diagnostic test only relying on the use of a single monoclonal antibody is likely to be asso-
associated with false-negative results. This may particularly be the case for a virus as variable as HIV-1. The value of the K14 IEIA for distinction of infections with different human lentiviruses possibly in combination with a monoclonal antibody to another epitope equally well conserved in HIV-1 strains should be studied further.

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References