VIROLOGICAL CHARACTERIZATION OF DUAL HIV-1/HIV-2 SERO-POSITIVITY AND INFECTIONS IN SOUTHERN GHANA

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***Conflict of Interest**: None*

SUMMARY

Background: Dual HIV-1/HIV-2 seropositivity (dual seropositivity) is common in West African countries including Ghana. The diagnosis of dual HIV-1/HIV-2 infections is however complicated as HIV-2 DNA is more often not detected in dual seropositive individuals.

Objectives: To detect the presence of HIV-1 and HIV-2 pro-viral DNA in dual seropositives and to determine the correlation between HIV-2 antibody titers and presence of HIV-2 DNA. The growth kinetics of HIV-1 and HIV-2 *in vitro* were also determined using plasma and lymphocyte cultures.

Design: Cross-sectional study

Setting: Urban and semi-rural HIV/AIDS clinics

Participants: 13 dual HIV-1/HIV-2 seropositives from Agomanya and Accra

Results: HIV-1 DNA was detected in uncultured peripheral blood mononuclear cells of all 13 patients but HIV-2 DNA in 4 (30.8%). HIV-2 antibody titres were not useful in determining the presence or absence of HIV-2 DNA (P=0.28, Mann-Whitney U test). HIV-2 specific antibody was detected in 12 of the 13 dual seropositives by peptide-inhibition, the only patient with an Innolia gp36 band rating of 1+ was shown not to be reactive. HIV-2 grew efficiently in the presence or HIV-1 *in vitro*.

Conclusion: HIV-2 DNA may not be detected in all dual seropositives thus not all of such patients may need drugs effective against HIV-2. Peptide based assays will be useful for correctly diagnosing dual seropositivity. Since HIV-2 may grow efficiently in the presence of HIV-1 and no commercial HIV-2 HIV RNA tests are available, dual seropositives on HAART need to be monitored to determine if a lack of immune restoration may correspond to an efficient suppression of HIV-1 RNA levels.

Keywords: HIV-1, HIV-2, dual, seropositivity, infection, Ghana

INTRODUCTION

In countries like Ghana which are situated in-between the initial African human immunodeficiency virus type 1 (HIV-1) and (HIV-2) epidemics, serologic reactivity to both HIV-1 and HIV-2 (dual seropositivity) is common.[1-4] Also, this serological profile has increased in previously HIV-2 endemic areas over the past decade.[5] The diagnosis of dual HIV-1/HIV-2 infections is however complicated as in most studies HIV-1 DNA is more commonly detected in dual seropositive individuals as compared to HIV-2 DNA.[6-9]

Assays based on synthetic peptides have provided a more specific distinction between HIV-1 and HIV-2 antibodies, as compared to Western Blotting.[10-12] Some studies have considered strong and equal intensities of the envelope transmembrane proteins of HIV (HIV-1 gp41 and HIV-2 gp36) on commercial blotting immunoassays to indicate dual seropositivity[6, 7] and the approach has been used to limit the effects of cross-reactivity. This criterion may however exclude true dual seropositives with low HIV-1 or HIV-2 antibody levels.

Despite having similar transmission routes, a slower heterosexual spread of HIV-2 as compared to HIV-1 has been observed[13]. Results from two independent studies have shown a slower progression to AIDS after HIV-2 infection as compared to HIV-1 infection[14, 15]. *In vitro*[6] and *in vivo*[7] studies have also suggested that HIV-1 often outgrows HIV-2 when they co-exist. It has been suggested that these differences are related to the biological behaviour of the viruses[16].

In order to characterize dual infections, we assessed the relation between humoral responses against HIV-2 and the presence of HIV-2 DNA in dual seropositive individuals in Ghana. An *in vitro* virological characterization was performed for patients with both HIV-1 and HIV-2 in culture.

PATIENTS AND METHODS

Patients

A cross-section of 188 patients at a semi-rural and an urban AIDS clinic from June to November, 1996 were enrolled for this study. After preliminary screening, blood samples were obtained from 13 of 23 dual HIV-1/HIV-2 seropositives who consented for a second blood sample to be taken. Ethical permission was obtained from Ministry of Health (Accra, Ghana), the University of Ghana Medical School (Accra), and Huddinge Hospital (Sweden), and informed consent was obtained from patients. All patients had their CD4 counts determined by FACS Count, Becton Dickenson, USA.

Serological Diagnosis

Anti-HIV seropositivity was determined using a rapid immunoassay (Target HIV-1/HIV-2, V-Tech. Inc., Pommona, California, USA). In brief, HIV antibodies were indicated by the appearance of blue spots at the sites where homologous synthetic peptides corresponding to the HIV-1 and HIV-2 transmembrane proteins were positioned. Confirmatory tests were done using Innolia (N.V Innogenetics, Antwerp, Belgium), according to the instruction of the manufacturer. This assay includes recombinant proteins and peptides for HIV-1 and HIV-2 antigens. The definition of dual seropositivity was based on the presence of the HIV-1 gp41 and the HIV-2 gp36 specific antibodies (Table 1).

HIV-2 Antibody Analysis

HIV-2 antibodies were semi-quantified using an assay based on a HIV-2 gp36 peptide (DQARLNSWGCAFRQVCHTTVPWV), and a protocol similar to that already described[17]. Plasma was diluted 1:100, followed by three-fold serial dilutions in 96-well microtiter plates (Nunc, Roskilde, Denmark). After incubation of the serum, antibodies were detected using alkaline phosphate-labelled goat anti-human IgG (Sigma Chemicals, St. Louis, MO) diluted to 1:1000 and alkaline phosphate substrate. Analysis was performed in triplicate using three separate plasma dilutions and optical densities read at 405 nm. Samples from eleven HIV-1 only and eight HIV-2 only seropositives were included as controls. The cut-off value was calculated for each plate using the mean value plus eight standard deviations of five HIV Swedish seronegative controls, which were analyzed in duplicate.

In order to analyze the specificity for the HIV-2 antibodies, an HIV-2 inhibition ELISA was performed which also included an HIV-1 gp41 peptide (A5 - DDDDQQLLGIWGCSGKLICTTAVPWN), corresponding to the HIV-2 gp36 peptide. In brief, plasma from patients were diluted at 1:100 and incubated at 37°C for one hour with decreasing concentrations of HIV-2 gp36 peptide (concentrations of 10000ng to 1ng in 50ul of serum dilution buffer) and also with dilution buffer only. A direct ELISA, using plates coated with the HIV-1 gp41 and HIV-2 gp36 respectively, was done as earlier described[17]. Analysis was performed in triplicate, using separate serum dilutions. The median of the two closest inhibition values (percentage) or the median was used.

Primary HIV cultures

Venous blood was obtained from the patients using the Vacutainer CPT, (Becton Dickenson, New Jersey, USA). Attempts to isolate HIV from plasma and peripheral blood mononuclear cells (PBMC) were done using phytohaemagglutinin-stimulated peripheral blood mononuclear cells (PBMC) of blood donors[18]. Cultures were kept for four to five weeks and supernatants were analyzed by an HIV-1 specific p24 antigen ELISA (Abbott Laboratories, North Chicago, Illinois, USA). After ten days of cultivation, cultured cells were harvested once weekly and frozen at -70°C and new PBMC was added.

Analysis of HIV-1 and HIV-2 DNA

DNA from uncultured PBMC and cultures from all 13 patients was extracted by phenol-chloroform and ethanol precipitation.

A semi-nested HIV-1 *gag* PCR was performed with primers and cycling parameters already described[18]. HIV-2 DNA was detected using primers[19] and a PCR protocol earlier described[20], but with a 50u1 mix and approximately 0.5ug DNA.

HIV-1 and HIV-2 DNA was detected in cultures of only two patients (3 and 6). For these patients, DNA extracts from weekly harvested cultured cells were quantified (DyNA Quant, Hoefer Pharmacia Biotech Inc., San Francisco, USA), and diluted to 10ng/u1. This was followed by six ten-fold serial dilutions. PCR was then performed from single dilutions in triplicate with positive controls.

RESULTS

*C*orrelation of PCR results with serology

HIV-1 DNA was amplified from uncultured PBMC of all 13 dual seropositive patients, but HIV-2 DNA was detected in only four (30.7%) subjects (Table 1). Details of the correlation between PCR and HIV-1 and HIV-2 antibody intensities have been shown in table 1.

The HIV-1 only seropositives reacted to the HIV-2 peptide ELISA with a maximum dilution of 1:300, so that was considered the minimum cut-off for HIV-2

specific antibodies. All eight HIV-2 only seropositives and all 9 dual seropositives had HIV-2 antibody titres above 1:300. All except one dual seropositive had HIV-2 specific antibodies (Table 1).

Growth of HIV-1 and HIV-2 in cultures

HIV-1 p24 antigen and HIV-1 DNA were detected in all plasma and PBMC cultures except the cultures for patient 12, and the plasma culture for patient 8. These cultures were excluded from the *in vitro* analysis.

HIV-2 DNA was detected only in the cells from the plasma and PBMC cultures of patient 3, and the PBMC cultures of patient 6. In contrast, HIV-1 DNA was detected in all cultures of these patients.

A semi-quantitative analysis of HIV-1 and HIV-2 DNA from patients 3 and 6 indicated that HIV-2 grew as efficiently as HIV-1 during the second to the fourth week of culture (Annex 1).

Table 1: Analysis of HIV-1 and HIV-2 antibody reactivity and the presence of HIV-1 and HIV-2 DNA in uncultured peripheral blood mononuclear cells obtained from 13 dual seropositive individuals

Patients	Age/Sex	CD4	Antibody Reactivity*		HIV-2 Ab titre[γ]	PCR using *gag* primers	
			HIV-1	HIV-2		HIV-1	HIV-2
1	55/F	447	>2+	3+	8100	+	+
2	56/M	NA	3+	2+	300	+	-
3	53/F	67	3+	3+	24300	+	+
4	38/M	98	3+	>1+	100	+	-
5	39/M	<50	3+	3+	218700	+	-
6	25/F	149	3+	3+	656100	+	+
7	30/F	432	3+	>2+	24300	+	-
8	33/F	384	3+	3+	24300	+	-
9 [ч]	24/F	297	3+	1+	300	+	-
10	31/F	62	3+	3+	72900	+	-
11	50/F	61	3+	3+	2700	+	+
12	16/F	555	3+	>2+	100	+	-
13	33/F	228	3+	3+	900	+	-

*Intensity of the antibody reactivity on the Innolia bands was scored: 1+, 2+, and 3+. Minimum requirements for dual seropositivity were 1+ –for both gp41 (HIV-1) and gp36 (HIV-2). > preceding integers means rating was greater than number, but less than the next successive integerr. [γ]HIV-2 antibody titres were determined with a peptide ELISA employing gp36 peptides. + and – indicate positive and negative results, respectively, NA means not available. [ч] An HIV-2 peptide inhibition assay showed that all except this patient mark were positive for HIV-22 antibodies. All patients were positive for HIV-1 gag PCR. HIV-2 antibody titers may not be useful in determining the presence of absence off HIV-2 DNA ($p=0.28$, Mann-Whitney U test).

DISCUSSION

Dual infection, as defined by the presence of both HIV-1 DNA and HIV-2 DNA, has been reported in different proportions of dual seropositive individuals in different studies.[6-9] In our study, HIV-2 DNA was detected in only four of 13 patients analyzed.

Although serologic cross-reactivity of HIV-1 antibodies and HIV-2 gp36 may wrongly classify patients as dual seropositive, our inhibition assay showed that only patient 9 was likely to be a false dual reactive. The low dilutions of plasma may have accounted for the cross-reactivity resulting in HIV-1 seropositives being reactive for HIV-2 specific antibodies (data not shown).

Our results are similar to a study by Ampofo et al[8] but in contrast to a recent study where HIV-2 was more commonly found in dual seropositives.[9] It is possible that the improvement of diagnostic assays over the years has made the new tests more specific for HIV-2 antibody detection.

A likely interpretation therefore is that these patients with low HIV-2 gp36 reactivity may have been exposed to HIV-2 without establishment of infection. The strict classification of strong and equal reactivity of gp41 and gp36 bands on commercial assays[6, 7] could therefore underestimate the true prevalence of dual seropositive individuals; those with HIV-2 antibodies irrespective of the concentration. There may be the need to develop in-house inhibition assays, which are cheap and reproducible to help to resolve the issue of dual serological reactivity in West Africa.

Although the number of subjects analyzed was small, there was no tendency for dual seropositive HIV-2 DNA positive patients to have a higher HIV-2 antibody titer than the HIV-2 DNA negative subjects. It was therefore not possible to use this criterion to single out dual infected patients. It has been suggested that a decrease of HIV-2 viral load may occur with disease progression[7]. However, three of the four dual infected patients had low CD4+ cell counts (Table1). It is possible that HIV-2 subtypes differences may account for this. A limitation of this study was that the time of infection with either HIV-1 or HIV-2 in the study patients was not known. Studies have shown that super infection with either HIV-1 or HIV-2 infection after the establishment of either infection is possible[21] and this could account for the presence of HIV-2 DNA even with very low CD4 counts.

HIV-2 also has similar replicative characteristics[22] and syncytium inducing capacities[23] as HIV-1. It has however been claimed that HIV-1 grew more efficiently than HIV-2 *in vitro*[6] and *in vivo*[7], and that these biological differences explain the more rapid epidemic spread of HIV-1[16], and the faster progression to AIDS in HIV-1 infected patients.[14, 15] Our *in vitro* analyses of the growth kinetics of the two types of viruses obtained from the same individual in a primary culture show that HIV-2 may sometimes grow efficiently in the presence of HIV-1. It is possible that the HIV-2 strains differences may exist that may influence the virulence of HIV-2 and that this may account for the observation made in our study.

In recent years, highly active antiretroviral therapy (HAART) has started in Ghana and HIV/AIDS patients are commonly put on two nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors (NNRTI). Since HIV-2 is not susceptible to NNRTI[24], there is a need for some dual seropositives to be put on protease inhibitors. Evidence already exists that mixed infection with HIV-1 and HIV-2 can be problematic during treatment.[25] Our results also indicate that specific antibody intensities of 1+ for HIV-2 gp36 on Innolia may not be reliable to confirm HIV-2 antibodies. Further studies will be needed to provide a screening algorithm that will correctly discriminate between HIV-1 and HIV-2 antibodies using rapid screening assays employing synthetic peptides.[9]

CONCLUSION

Discrimination between HIV-1 and HIV-2 antibodies can be better achieved using peptide-inhibition assays and not all HIV patients with HIV-1 and HIV-2 antibodies are actively infected with both viruses. Since the guidelines for antiretroviral therapy considers dual infections as a special condition warranting the use of protease inhibitors[26], a screening algorithm considering the fact that not all dual seropositives have HIV-2 DNA needs to be established. Follow-up of dual seropositives currently on a HAART regimen with NNRTI needs to be done to determine if some may have CD4 cell levels continuously suppressed suggesting efficient growth of HIV-2 in spite of treatment.

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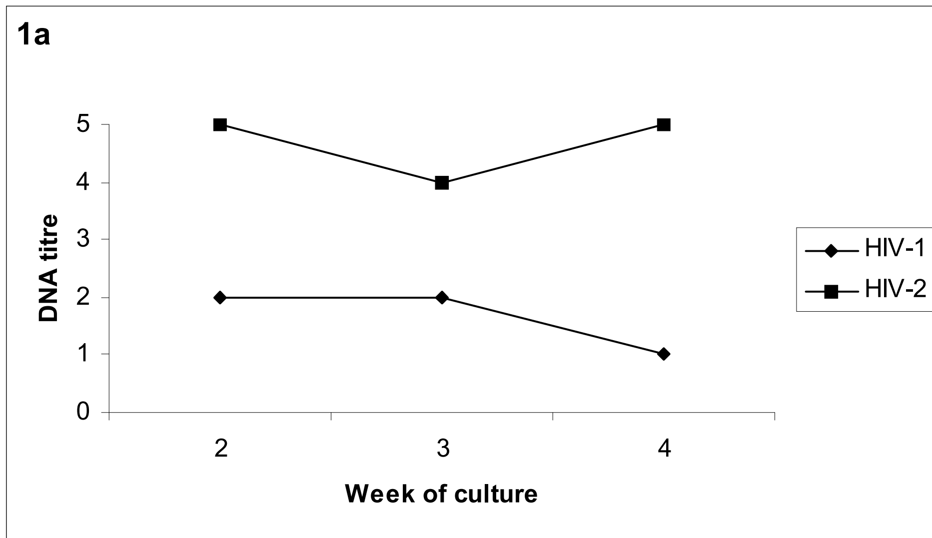

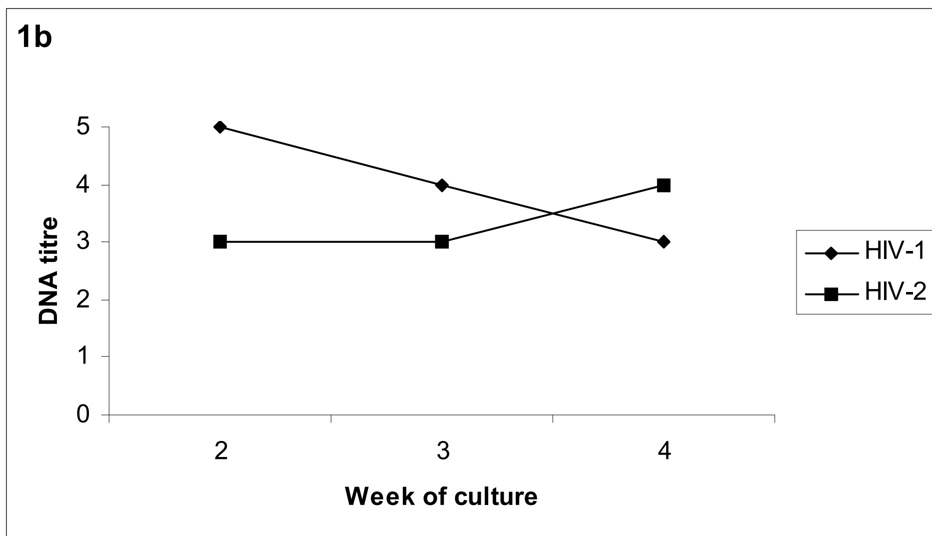

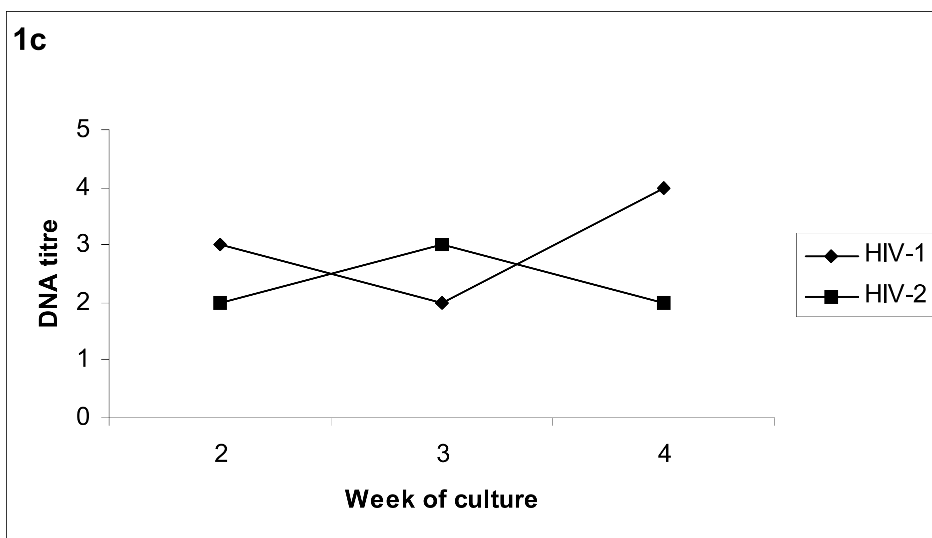

Annex 1: Levels of HIV-1 and HIV-2 DNA from lymphocyte and plasma cultures of patient 3 (1a, 1b) and lymphocyte culture of patient 6 (1c). HIV-1 and HIV-2 DNA titers were detected using PCR for the *gag* regions. 1 corresponds to a genomic DNA of 10ng/µl, 2 is a tenfold dilution of 1, 3 is a tenfold dilution of 2.