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CHARACTERIZATION OF AFRICAN HUMAN RETROVIRUSES RELATED
TO HTLV-III/LAV

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FOREWARD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 86-23, Revised 1985).

For the protection of human subjects the investigators(s) have adhered to policies of applicable Federal Law 45CFR46.

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Our recognition of the existence and properties of exogenous human retroviruses has increased dramatically in the last decade. After the identification of HIV-1 as the causative agent of AIDS, we have gained a new appreciation of the unique and complex properties of this pathogenic human retrovirus. Early in 1985, a related virus, Simian Immunodeficiency Virus (SIV, previously termed STLV-3) was found in immunodeficient macaque monkeys. SIV was found to have major viral antigens that were similar and cross-reactive with the viral antigens of HIV-1 (1). SIV is known to induce immunosuppression in the Asian macaque monkey, however, in its natural host, the African Green monkey it appears to be relatively nonpathogenic (1-3). The existence of a primate relative of HIV-1 found in high numbers of naturally infected African primates suggested the possibility that people might also be susceptible to infection with an SIV related virus.

In 1985, a new human T-lymphotropic retrovirus was discovered in Senegal, West Africa (4). We described antibody reactivity in healthy Senegalese that demonstrated strong antibodies to the env, gag and pol antigens of SIV. These same samples when reacted with HIV-1, only showed weak cross-reactive antibodies to the gag and pol antigens. It was therefore, recognized that these individuals had been exposed to a virus more closely related to SIV and more distantly related to the prototype AIDS virus, HIV-1 (4,5). This new human retrovirus has now been termed Human Immunodeficiency Virus Type 2 (HIV-2) (6). Various strain names have been given to HIV-2, including: HTLV-4, LAV-2, SBL-6669, HTLV-4 (ST), it is now believed that these are all the same virus type. All HIV-2 strains thus far identified are serologically cross-reactive

and therefore serology-based studies are not thought to be strain-specific (7-9).

The studies funded by this contract have focussed on characterizing the biologic effects of HIV-2. This has included virologic studies to analyze the major viral antigens and compare them to analogous antigens of HIV-1, HIV-2 cell tropism studies and superinfection studies with HIV-1. The proposed animal inoculation studies will provide us with information on the *in vivo* effects of HIV-2 infection in the chimpanzee model and the interactive effect of this virus with HIV-1. The most central portion of our studies has been to study the biology of HIV-2 infection in infected people in Africa.

The antigenic relatedness of both SIV and HIV-2 to the prototype HIV virus prompted both the discovery and further classification of these related viruses (1,4). Subsequent genetic analysis has shown HIV-2 to be most closely related to SIV (less than 20% difference) and more distantly related to HIV-1 (approximately 50% difference) (10-12). Similar to HIV-1, these related viruses demonstrate tropism to the T4 lymphocyte (1,5,13). The overall genetic organization of all three virus types are similar with the exception of a unique open reading frame termed "X" found in both HIV-2 and SIV (10-12).

The major viral antigens of HIV-2 have been identified by immunoblot and radioimmunoprecipitation analysis; they bear striking similarity and cross-reactive epitopes with the viral antigens of HIV-1 (4,5). The gag encoded products include a p55 myristylated precursor, a major core protein, p24-

26, and an amino-terminal myristylated gag protein, p15 (4,5,14). The pol-encoded proteins, readily distinguished by immunoblot and radioimmunoprecipitation of virus preparations include a p64, p53 and p34 (endonuclease) (5,15). The most highly immunogenic antigens are the env-related glycoproteins which includes a gp160 precursor, the mature envelope protein the gp120 and the transmembrane gp32-40. There appears to be polymorphism in the env-related glycoproteins, similar to what was reported for different strains of HTLV-1 (4,5,8,16).

The gag and pol genes are well conserved for both HIVs and SIV (10-12). The gag and pol encoded proteins exhibit broad serological cross-reactivity. It is the presence of gag and pol antigens in various HIV-1 serologic assays that enables the frequent detection of HIV-2 antibodies which are cross-reactive to those conserved epitopes. The env genes of HIV-1 and HIV-2 show less conservation at a genetic and antigenic level, whereas HIV-2 and SIV show a high degree of conservation (10-12). Sequence analysis of several HIV-2 and SIV strains have demonstrated a stop codon in the middle of the open reading frame encoding the transmembrane protein (11,12,17); this corresponds to the smaller transmembrane protein size that is seen with certain HIV-2 isolates (16). HIV-2 isolates SBL-6669 and HIV-2(ST) are reported to have two smaller molecular weight glycoproteins that are thought to correspond to two different size transmembrane proteins, a gp32 and a gp40 (8,16). It is not known whether these two glycoproteins are in fact transmembrane proteins. If so, the presence of two different sized transmembrane proteins may indicate that these cell lines contain a mixture of virus strains or one virus strain capable of modulating expression of the transmembrane stop

codon. It is still to be determined if the stop codon is seen in viruses in vivo or if it represents an in vitro artifact. If the former, it will be important to determine if potential modulation of the stop codon can affect the functional properties of this virus. Nucleotide sequence comparison between HIV-1 and HIV-2 or SIV have revealed conserved regions scattered throughout the envelope gene (11,12). It is still not known whether these regions will correspond to immunogenic domains capable of eliciting a cross-protective response to all virus types. Although in limited studies it has been shown that HIV-2 positive sera is capable of neutralizing various HIV-2 strains and additionally neutralizing some HIV-1 strains at lower titer, whereas the neutralizing response of HIV-1 infected individuals appeared to be type-specific (18).

Molecular studies have demonstrated the presence of six accessory genes with variable homology to the HIV-1 genes (sor, tat, art/trs, 3'orf/F, R, and X). Functional evidence of tat activity has been shown for HIV-2 and SIV-2, similar and cross-reactive with HIV-1 tat (19,12). The function and immunogenicity of the other accessory genes is still under study. Comparative analysis of these regulatory genes' function and correlation to the biological effects of these related viruses may provide important clues on the pathogenicity of HIV-1.

HIV-2 was given its name to indicate its close relationship to HIV-1 this was based on similar cell tropism, antigenic and genetic properties (6). However, the comparative ability of HIV-2 to induce immunodeficiency is still under active study. Preliminary surveillance studies have already demonstrated significant rates of HIV-2 infection in West Africa.

Therefore, it is of critical importance to determine the clinical significance of HIV-2 infection and evaluate its potential as a second AIDS causing virus.

The health status of individuals from which virus has been isolated is but one means of assessing the pathogenic potential of a virus. HIV-2 isolates have been made from healthy individuals and AIDS patients originating from West Africa (4,8,13). Clavel and coworkers (20) have described HIV-2 infected West African patients admitted for treatment to a hospital in Lisbon, Portugal. The clinical picture of endstage AIDS patients found to be infected with HIV-2 has been similar to that of HIV-1 induced AIDS. In follow-up clinical studies, described by Brun-Vezinet and colleagues (21), 2 of 3 HIV-2 positive AIDS cases were still alive and stable three years after the diagnosis of AIDS. This seemed to indicate a possible difference in the pathogenicity between HIV-1 and HIV-2. Unfortunately, it is difficult to adequately assess the pathogenic potential of any suspected agent with isolation of select disease patients alone.

In West Africa, seroepidemiologic surveys (n=15,652) conducted in major urban areas of Senegal, Guinea, Guinea Bissau, Mauritania, Burkina Faso, Ivory Coast, Gambia, Cape Verde and Benin (Table 1) have demonstrated moderate to high rates of infection with HIV-2 in all countries surveyed except Mauritania (22-24). For these studies, serologic diagnosis was accomplished by immunoblot and/or radioimmunoprecipitation analysis with both HIV-1 and HIV-2 antigens. In general, the prevalence of HIV-2 was higher than that of HIV-1 in any West African country surveyed when control healthy or sexually active risk groups were examined. However,

HIV-1 was more frequent in the few AIDS patients or suspect cases that were examined; these individuals were frequently of Central African origin or had a history of recent travel in Central Africa (16).

In the countries surveyed the HIV-2 prevalence in healthy adult populations varied from 0.2-9.2% whereas rates of HIV-1 were much lower. Limited studies conducted in select rural populations of Senegal have failed to demonstrate comparable rates (0%) of HIV-2 seropositives when compared to urban populations, indicating that HIV-2 infection may be more predominant in urban settings as compared to rural settings (16). Similar observations have been made with HIV-1 in Central African populations, where lower and more stable seroprevalences of HIV-1 are found in rural populations (25). Almost 80% of sub-Saharan Africa resides in such a rural setting, it will therefore be of paramount importance to better define the modes of HIV transmission between these distinct populations.

HIV-2 prevalence was higher in sexually active risk groups such as female prostitutes and sexually transmitted disease patients when compared to healthy control populations (22-24). In some urban centers of West Africa the prevalence for HIV-2 in female prostitutes ranged from 15-64%. All individuals were examined at the time of the serum sampling and were found to be without signs or symptoms of AIDS. The prevalence ratio of sexually active risk groups compared to control populations was 7.4 ($\chi^2=297$, $p<0.0001$) Therefore, HIV-2 appears to be sexually transmitted like HIV-1 and in Africa this is thought to be primarily heterosexual transmission.

Large-scale seroepidemiologic studies allow for the evaluation of large numbers of individuals of varying health status and its potential association with virus. In our studies, there was no significant difference between the prevalence of HIV-2 in immunosuppressed individuals or AIDS patients when compared to healthy controls from the same geographic locale (22-24). Since, it is recognized that AIDS in West Africa is still relatively infrequent, we investigated the possibility that some other associated disease entity might better illustrate the potential immunosuppressive properties of HIV-2. It has been well-recognized from numerous studies in Central Africa that tuberculosis is highly associated with HIV-1 infection and AIDS (26).

40-70% of tuberculosis cases examined in Zaire were shown to be HIV-1 seropositive. We therefore hypothesized that since tuberculosis was also endemic in West Africa, this population might also show an increased association with HIV-2 seropositivity. In Senegal, Ivory Coast and Guinea Bissau (n=345) there was no significant difference between the seroprevalence in control populations compared to that in tuberculosis patients (22). This is in marked contrast to what is seen in comparable studies in Central Africa with HIV-1; indicating a striking difference in the pathobiology of HIV-2 from that of HIV-1.

HIV-2 is similar to HIV-1 in its tropism for CD4+ lymphocytes, its effects on CD4 expression and the ability of externalized envelope proteins to complex directly with the CD4 molecule (13,27). It is therefore of interest to determine if previous HIV-1 or HIV-2 infection of CD4+ lymphocytes will preclude infection with the other virus type. Such virus-

attachment interference has been previously demonstrated in other retrovirus systems, most notably Rous sarcoma virus (28). In the RSV system, chick cells infected with a noncytotoxic, nontransforming avian leukovirus abrogates the cellular receptors for RSV, but has no effect on the susceptibility of the cells to infection by other unrelated viruses. Interference appears to be due to blockage of the cellular receptors which are specific for viruses of each of the antigenic virus types.

We have conducted preliminary experiments to address this question. Five million persistently infected HIV-2(289) Hut-78 cells, with 100% antigen expression by immunofluorescences, were superinfected with 1) 1.0ml of filtered supernatant from HIV-1(3B) infected Molt 3 cells and 2) 4.0 ml of filtered supernatant from HIV-1(3B) infected Molt 3 cells. Five million uninfected Hut-78 cells were infected with 1.0ml of filtered supernatant from HIV-1 (3B) virus as controls. All cultures were standardized for the same concentration of cells per culture media and monitored at days 3,7,10, 15 and 19 for cell concentration and radioimmunoprecipitation (RIP-SDS/PAGE) of HIV-1 and HIV-2 antigens. The RIP-SDS/PAGE assay in our hands, requires approximately 40% antigen expression by immunofluorescence to visualize viral-specific bands. The results are given on figures 1a and 1b.

Persistently infected HIV-2 cells were successfully superinfected with HIV-1 virus by day 7 after virus inoculation. On day 7, viral antigens of both HIV-1 and HIV-2 were readily apparent by RIP-SDS/PAGE. Slight differences in the electrophoretic mobility of the viral antigens of HIV-1 and HIV-2 as well as monospecific sera allowed for the distinction in the

types of viral antigens expressed. Although superinfection of the Hut-78 cells and subsequent co-expression of both viruses was demonstrated, the apparent cytolytic effect of acute HIV-1 infection appeared to be diminished with low concentration of virus (1.0 HIV-1) and totally abrogated at high concentration of virus (4.0 HIV-1). Our interpretation of these preliminary results includes the possibility that prior infection CD4+ cells may provide some protective effect to the acute cytolytic properties of HIV-1 virus although virus-attachment interference does not appear to occur. It is also possible that a small number of cells in our persistently HIV-2 infected culture were expressing CD4 and were therefore available for HIV-1 infection. This appears unlikely since virtually all the cells were immunofluorescent positive for HIV-2 antigens and CD4 was not detectable. Furthermore, since the RIP-SDS/PAGE assay requires a significant proportion of the cells to be expressing specific viral antigens (>40%), the expression of HIV-1 proteins by day 7 post-infection is highly suggestive of superinfection. Further studies are necessary to verify these results and to also assess the ability of HIV-2 virus to superinfect HIV-1 infected cells.

In Burkina Faso and Ivory Coast significant rates of infection with both HIV-1 and HIV-2 are seen in risk populations (22-24). In these countries a number of individuals were found who possess antibodies with strong reactivity to the env antigens, particularly the gp120, of both HIV-1 and HIV-2 (22,23). This type of serologic profile may be indicative of either infection with an intermediate type of virus, double exposure to both HIV-1 and HIV-2 or unusually high-titer cross-reactive antibodies to both. Isolation of both HIV-1 and HIV-2 has not been demonstrated to date

(22,23,29). It is therefore not known if some individuals may have been exposed to both viruses but maintain persistent infection with only one type. Or, if previous infection with one virus type may induce some cross-protective immunity and/or interference phenomenon.

In many parts of the world the potential for infection with both HIV-1 and HIV-2 may exist in certain risk populations. It is not known what the interactive effect of HIV-1 and HIV-2 will be in vivo. Prospective follow-up studies with virus isolation and characterization as well as clinical monitoring will determine if interference, double infection or recombination with these viruses occurs and its effect.

Preliminary studies on the hematological and immunological status of HIV-2 infected prostitutes in Senegal have been completed (31). Generalized lymphadenopathy and clinical symptomology of AIDS was not present. Comparison to seronegative prostitutes and minor surgery patients were made and significant elevations were seen in T8 lymphocytes ($p=.03$), IgG ($p=.001$) and B2-microglobulin ($p=.03$). The mean T4 lymphocyte count in seropositive prostitutes was lower than in seronegative prostitutes (757 vs 1179, $p=.15$), but this difference was not statistically significant and appeared to be correlated with age. No significant differences were noted between seronegative and seropositive prostitutes in blastogenic response to various mitogens. Antilymphocyte antibodies above background were not present in either population.

Clinical examination of over 62 HIV-2 infected West African prostitutes has failed to demonstrate an increase in AIDS-related signs or symptoms or

generalized lymphadenopathy (30). This is in contrast to similar studies conducted on Nairobi prostitutes with HIV-1 infection, where 54% of the seropositive prostitutes were found to have generalized lymphadenopathy on physical examination (31). In Rwandese HIV-1 prostitutes, clinical examination revealed 83% generalized lymphadenopathy and 38% signs or symptoms suggestive of HIV-like disease (32).

We have continued to collect data on our prospective prostitute cohort study. These are a subset of the larger population described above. All HIV-2+ prostitutes were matched with seronegative prostitutes (1:2 matching ratio) by age, by nationality and by initial year of registration in the clinic (33).

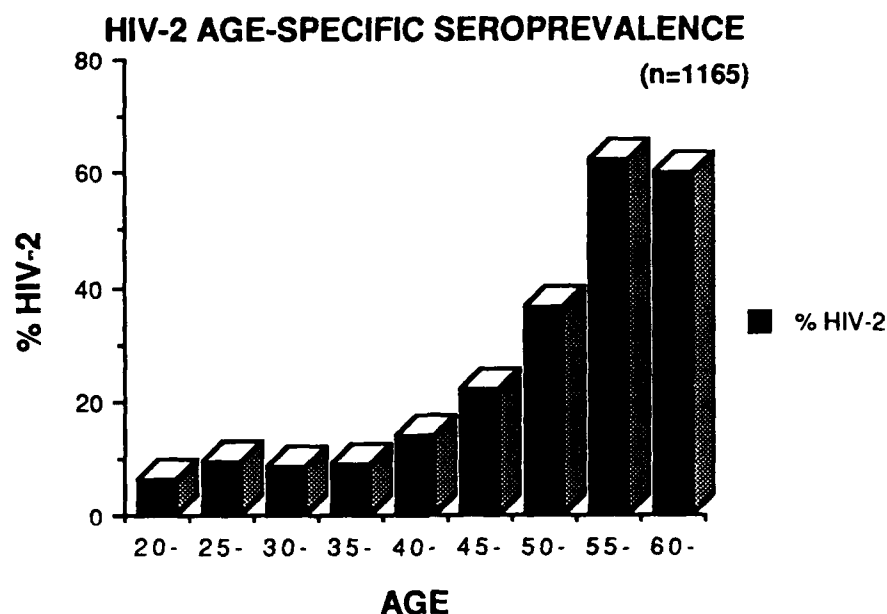
From the date of known serologic status, 92 HIV-2 seropositive prostitutes have been matched with 180 seronegative prostitutes.

	Mean Age	Mean Years in clinic	Mean Person-years observed
HIV-2+ N=92	37	6.8	94
Negative N=180	36	6.5	268

There has been no generalized lymphadenopathy (>1cm in two sites), oral candidiasis, or ARC signs or symptomatology in either group. The overall incidence rates of medical problems between the groups has been similar. This data combined with evaluation of immunosuppressed individuals from the area indicates a difference in pathobiology of HIV-2 from HIV-1.

It therefore appears that the natural history and clinical course of HIV-2 infected individuals may vary significantly from that of HIV-1. It is yet to be determined, if the biology of HIV-2 will differ from HIV-1 only in its latency and incubation period.

Despite the lack of association of HIV-2 with AIDS or related disease groups it is still possible that this T-lymphotropic retrovirus is capable of inducing disease but perhaps with a long latency and decreased attack rate. The lack of abnormal clinical findings in HIV-2 infected individuals could be explained by a very recent introduction of this retrovirus into the population. Retrospective studies conducted in populations of Dakar, Senegal in the mid-1970's demonstrate that HIV-2 was present at least 18 years ago. Furthermore, we have found that the age-specific seroprevalence to HIV-2 in seropositive female prostitutes in Dakar increases with age (9). In prostitutes over the age of 50 the HIV-2 seroprevalence rate was close to 100%. This age-specific seroprevalence curve is indicative of an endemic virus present in the population for at least several generations. This is consistent with the relatively high rates of HIV-2 infection found in healthy adult control populations.

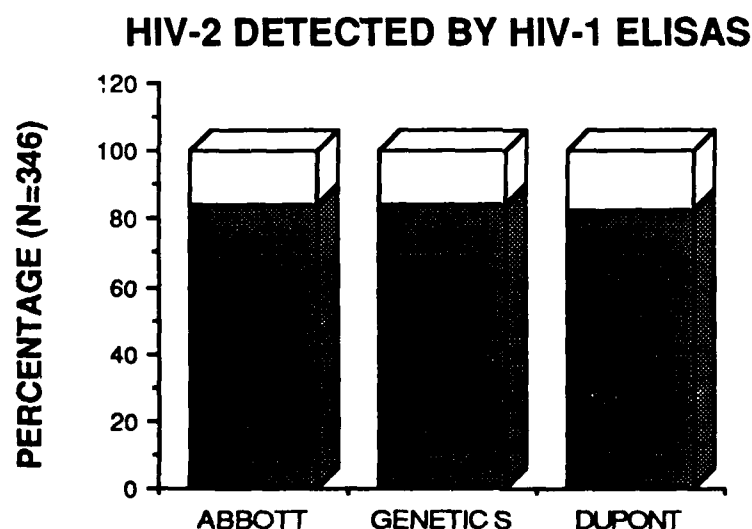


Therefore, the results of epidemiologic and prospective clinical studies indicate that HIV-2 is not identical to HIV-1 in pathogenicity as was once reported. Further follow-up studies are necessary to define the natural history and clinical significance of HIV-2 infection.

HIV-2 Distribution Outside of West Africa

HIV-2 infection at the present time appears to be most concentrated in West Africa. Serologic surveys conducted in other African nations such as Zaire, Burundi, Tanzania, Zambia, Kenya, Cameroon, Congo, Equatorial Guinea, Tchad, Ethiopia, Angola and Malawi failed to demonstrate evidence of HIV-2 infection (34-37), despite variable levels of HIV-1 infection. In many of these areas, HIV-1 infection and AIDS is quite high, however there were no cases of HIV-2 seropositivity (34).

Rare cases of HIV-2 infected individuals have been detected in Europe usually in individuals with connections to West Africa (38-40). Studies by the French Society of Blood Transfusion surveyed 100,114 blood donors and found .02% HIV-1 and 0% HIV-2. Limited studies conducted in the United States failed to identify HIV-2 in groups of prostitutes, homosexuals and Haitians, although it may be that the risk groups for infection with this virus may differ from that of HIV-1 (41,16). It is likely that increased international travel will no doubt enhance the spread of HIV-2 outside of West Africa. With this recognition, the screening of US blood donors for HIV-2 has been raised. We have recently conducted a study to determine the ability of the HIV-1 screening ELISAs presently used in blood banks. 346 western blot confirmed HIV-2 positive samples from West Africa were tested on Abbott, Genetic Systems and Dupont HIV-1 ELISAs. 82.4%-84.1% of the HIV-2 positive samples were detected by all three of these HIV-1 ELISAs (see below).



Only 12/346 (3.4%) of the HIV-2 positive samples were negative on all three HIV-1 ELISAs examined. It can be predicted that the present rates of HIV-2 in US blood donor groups would be quite low, presumably in the range of .0001-.0000001%. The currently employed HIV-1 ELISA assays could be expected to detect at least 80% of the HIV-2 positives without employing a second screening assay. Better estimates of the prevalence of HIV-2 in blood donors as well as the identification of risk groups in the US will be important prior to policy decisions on blood bank screening for HIV-2.

TABLE 1: HIV-2 SEROPREVALENCE IN WEST AFRICA BY HEALTH CATEGORY

COUNTRY	TESTED #	CONTROL	RISK	HOSPITAL	AIDS
SENEGAL	10,424	0.5%	12.8%	1.0%	6.5%
GUINEA	458	0.9%	0%	1.5%	--
GUINEA BISSAU	463	9.2%	64.0%	13.6%	--
MAURITANIA	356	0%	0%	0%	--
BURKINA FASO	1,106	0.2%	17.2%	1.7%	--
IVORY COAST	1,376	4.0%	20.0%	5.0%	5.4%
BENIN	923	0.3%	3.5%	0%	--
CAMEROON	46	0%	0%	0%	--

Figure 1a: HIV-1 superinfection of HIV-2 infected Hut-78 cells.

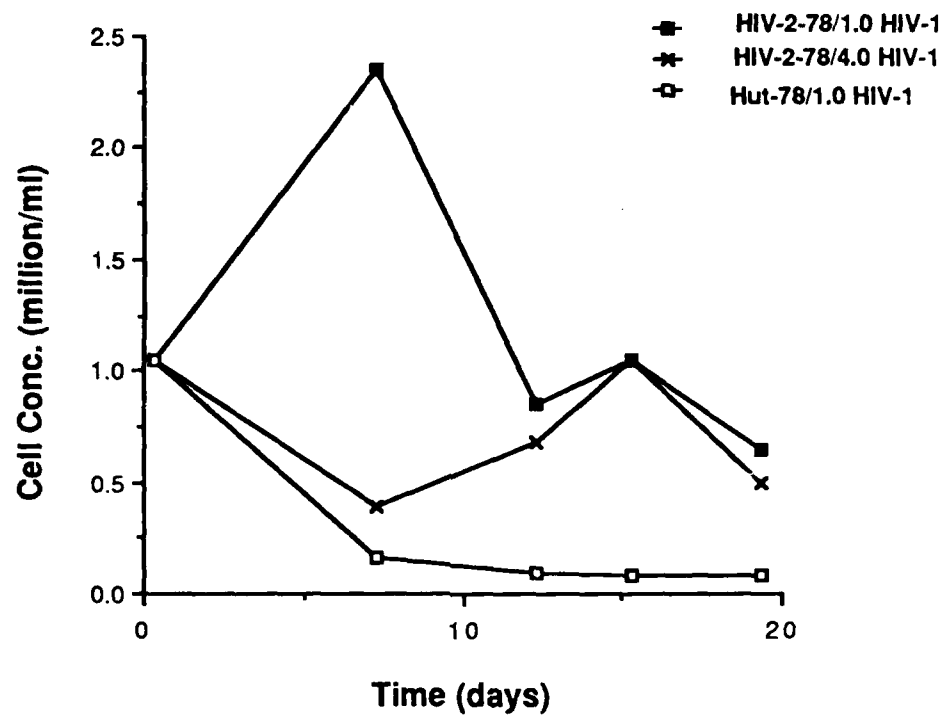


Figure 1b: Viral Protein Analysis of HIV-1 superinfection of HIV-2 infected cells by RIP-SDS/PAGE.

<u>DAY</u>	<u>CULTURE</u>	<u>HIV-2</u>		<u>HIV-1</u>	
		<u>env</u>	<u>gag</u>	<u>env</u>	<u>gag</u>
3	Hut-78/1.0 HIV-1	-	-	+	+
7	Hut-78/1.0 HIV-1	-	-	+	+
16	Hut-78/ 1.0 HIV-1	-	-	+	+
3	HIV-2-78/1.0 HIV-1	+	+	-	-
7	HIV-2-78/1.0 HIV-1	+	+	+	+
16	HIV-2-78/1.0 HIV-1	+	+	+	+
3	HIV-2-78/4.0 HIV-1	+	+	-	+
7	HIV-2-78/4.0 HIV-1	+	+	+	+
16	HIV-2-78/4.0 HIV-1	+	+	+	+

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