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HSV-2 Infection in Potential High Risk Group Volunteers for HIV Vaccine Trials

B Bekan Homawoo, M Coldiron, E Tekirya, J Bizimana, E Karita, S Allen and RZHRG

Background: HSV-2 infection has been associated with transmission and acquisition of HIV and a more rapid progression of HIV disease. HSV-2 is highly prevalent in sub-Saharan Africa, though it is often asymptomatic. New HIV infections frequently occur in discordant couples who remain a high risk group and the HIV negative partners in those couples are potential volunteers for phase III HIV vaccine trials.

Objective: To establish HSV-2 prevalence amongst HIV discordant couples and the possible impact of that prevalence on the measurement of efficacy trials' endpoint.

Methods: At Projet San Francisco (PSF) in Kigali, we conducted a cross-sectional study to establish the prevalence of HSV-2 in 729 discordant couples using HSV-2 Focus ELISA. We considered an

Index Value of >3.5 positive and presumptive for the presence of IgG antibodies to HSV-2, an Index Value of <0.90 negative, and an Index Value of ≥ 0.90 but ≤ 3.5 equivocal. Equivocal results were not included in this analysis.

Results: The analysis of our primary data shows that HIV positive individuals have a higher prevalence of HSV-2 (79%) compared to HIV negative (59%). In addition, HIV positive females are more likely to be HSV-2 co-infected (84%) compared to HIV positive males (73%). Moreover, the HIV negative partners of HIV/HSV-2 co-infected individuals have higher risk for HSV-2 infection compared to partners of HIV-infected individuals not HSV-2 infected (OR 4.4, 95% CI 1.8-10). We also looked at CD4 count as a measure of HIV disease progression. The mean CD4 count was lower among HIV/HSV-2 co-infected individuals (480 cells/ μ l) compared to HIV positive HSV-2 negative individuals (505 cells/ μ l), but this difference was not statistically significant.

Conclusion: HSV-2 is highly prevalent in both partners of HIV discordant couples. HIV vaccine trials conducted in high risk groups should take into account the potential confounding effect of HSV-2 infection in both the donors and the recipients given the association between HIV AND HSV-2 infections. HSV-2 testing should be advocated as standard of care for those couples in loom of an HIV vaccine.

Qualifying a vaccine trial laboratory in Rwanda for PBMC isolation, cryopreservation and shipping

J Bizimana, E Karita, MJ Boaz, T Tarragona, J Gilmour; J Stout, E Shutes, E Tekirya, V Musengamana, E Hunter and S Allen

BACKGROUND: Successful development of an AIDS vaccine requires quality-assured, robust data pivotal for evaluating vaccine safety and immunogenicity. Prior to initiation of a clinical vaccine trial with centralized immunogenicity testing, participating immunology laboratories must be competent in isolation, cryopreservation and shipment of PBMCs, thus ensuring vaccine immunogenicity can be assessed.

METHODS: In preparation for an IAVI sponsored Phase I HIV vaccine trial, Projet San Francisco, Rwanda, in collaboration with IAVI, developed an immunology laboratory. Staff were recruited, equipment was installed and training provided on site and in London. The laboratory was enrolled into accreditation and QA programs, part of the IAVI accreditation procedure was evaluation of site competence in PBMC isolation. An IAVI qualifying run was undertaken in which blood was drawn from four healthy volunteers and using IAVI Core Laboratory SOPs, PBMCs were isolated, cryopreserved and shipped in liquid nitrogen to the IAVI Core Laboratory, London.

RESULTS: Each of the five criteria for passing the IAVI qualifying run was satisfied. At the site in Rwanda, the yield of PBMC isolated per ml of blood for each volunteer ranged from 0.9 to 1.5 million cells. The recovery of PBMCs in London after overnight rest, determined using an automated cell counter, ranged from 59-123%, with viability of 96-97%. No red blood cell contamination was evident. Assessment of PBMC performance in a qualified IFN γ ELISPOT assay further showed good quality in all samples: low backgrounds (<37.5 SFC/106), positive responses to PHA (>949 SFC/106), and to the physiologic control flu, EBV and CMV peptides. Initiation of the IAVI V001/PAVE

vaccine trial in Rwanda has continued to demonstrate the competence of the Rwanda site and laboratory in PBMC isolation, cryopreservation and shipping.

CONCLUSION: Following training and site development with IAVI, the Projet San Francisco site in Rwanda successfully passed the IAVI qualifying run showing competence in PBMC isolation, cryopreservation and shipping. This was one critical aspect in validating the site to conduct an HIV vaccine trial. Subsequent initiation of an HIV vaccine trial in Rwanda has provided further confirmation for the quality of this site laboratory to perform within HIV vaccine trials.

Evidence for Potent Autologous Neutralizing Antibody Titers and Compact Envs in Early Infection with Subtype C Human Immunodeficiency Virus type 1

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Background: Information about neutralizing antibody responses in subtype C infected individuals is limited, even though this viral subtype causes the majority of AIDS cases worldwide.

Methods: The course and magnitude of the autologous neutralizing antibody (Nab) response against viral envelope (Env) glycoproteins present during acute/ early infection with subtype B and C HIV-1 was compared. Nab responses were evaluated in 6 subtype B infected and 11 subtype C infected subjects over a mean evaluation period of 25 months using a pseudovirus reporter gene assay. All subjects in the C cohort were infected through heterosexual contact, while 5 of the 6 subjects in the B cohort were infected via male -to-male contact.

Results: The kinetics and magnitude of the Nab responses varied among subjects in the B and C cohorts; however, the median IC50 titer reached by antibody in the plasma of subtype C infected subjects, overall, was 3.5 -fold higher than in the subtype B infected subjects ($p=0.06$). The higher titers of Nabs in the C cohort were associated with viruses having significantly shorter amino acid length ($p=0.002$) in the V1 -V4 region of the surface Env glycoprotein, gp120, compared to the B cohort. Despite the potency of the autologous subtype C Nab response, it was not directed against cross-neutralizing epitopes.

Conclusions: These data demonstrate that subtype C Envs elicit a potent yet restricted Nab response early in infection that frequently reaches IC50 titers in excess of 1:1,000 and suggest that clade - specific differences may exist in Env immunogenicity or susceptibility to neutralization.

Correlates of Neutralization Resistance in Heterosexual Transmission Pairs infected with Subtype C Human Immunodeficiency Virus type 1

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Background: Potent autologous antibody -mediated neutralizing responses are produced against HIV-1, but are thwarted by successive escape variants that remain one step ahead of the humoral response.

Resistance to neutralization is achieved through adaptations that occur in the envelope (Env) glycoproteins as the viral quasispecies expands. We previously reported that the HIV-1 quasispecies passes through a genetic bottleneck during heterosexual transmission in Zambia that selects for compact viruses with high sensitivity to neutralization by antibodies in the infecting partner's plasma. This suggests a potential relationship between the topology of the newly transmitted Envs and their neutralization sensitivity.

Objectives: We investigated whether the sequence diversity, length, or glycosylation of the gp120 V1-V4 region was correlated with neutralization sensitivity.

Methods: A statistical analysis was performed to determine whether sequence adaptations in the V1V4 region of gp120 were linked to neutralization sensitivity. V1V2 and a2 helix chimeras were constructed to probe the role of these regions in neutralization phenotype.

Results: Sequence divergence and acquisition of length in V1V4 were correlated with a decrease in sensitivity to neutralization. Exchange of the V1V2 domain between neutralization resistant donor Envs and a neutralization sensitive recipient Env confirmed that expanded V1V2 domains confer neutralization resistance. A mutual information analysis revealed 9 amino acid positions in V1V4 that were significantly associated with neutralization phenotype. Five of these 9 positions were located in a helical structure (a2) on the outer domain of gp120 that is under strong positive selection in subtype C infection. However, exchange of the a2 helix domain between neutralization-resistant donor Envs and a neutralization-sensitive recipient Env did not alter the neutralization sensitivity phenotype.

Conclusions: These results argue that the adaptations that facilitate resistance against neutralization in subtype C viruses, such as increased V1V4 length, may diminish the capacity to establish a new infection. Some sequence adaptations, particularly those in a2, appear to play a role in decreased sensitivity to neutralization but do not directly confer this phenotype. The influence of the a2 helix on neutralization sensitivity suggests that subtype C viruses evade the humoral immune response through a different mechanism than subtype B viruses.

Seasonal Variation in Clinical Laboratory Parameters among HIV-Uninfected Adults in Kigali, Rwanda

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Background: Clinical laboratory reference ranges have not yet been established in many African countries. Studies have shown differences in laboratory values between African and US or European populations, possibly due to endemic diseases, genetic, nutritional, environmental, and/or socioeconomic factors. There is seasonal variation of several diseases, such as malaria that can affect laboratory values, however the impact of the seasonal changes on laboratory parameters has not yet been assessed in Sub-Saharan Africa.

Objective: To assess the impact of the seasonal changes on laboratory parameters among HIV-uninfected adults.

Methods: 400 clinically healthy, HIV-uninfected adults (200 women and 200 men), aged 18 to 53 years were enrolled in Kigali, Rwanda between December 2004 and May 2005 (rainy season). After providing informed consent (written/thumb print) all volunteers underwent a comprehensive medical history, a complete physical examination and evaluation of laboratory values (chemistry and hematology). They were then invited to come back to the clinic during the dry season (June – September 2005).

Results: The mean age was 28.5 years for females (range: 18-50), and 32.4 years for males (range: 20-53). 37 volunteers (19 females and 18 males) were excluded from the first analysis (rainy season), and an additional 29 (19 females and 10 males) excluded from the second (dry season). The most frequent reasons for exclusion were hepatitis B/C seropositivity (28 cases) and pregnancy (18 cases). There were no differences across seasons for creatinine and SGOT/ALT values. SGPT/AST, Hemoglobin, and CD4 counts were higher in the rainy season, and these differences were statistically significant. However, the absolute differences were modest, and their clinical significance was negligible. The median values for SGPT were 20 iU/L in the rainy season and 18 iU/L in the dry season ($p < .0001$). Corresponding values for CD4 counts were respectively 932 and 877 cells/ μ L ($p < .0001$).

Conclusion: There were no observed clinically significant variations across seasons in laboratory parameters at this site, suggesting that enrollment into clinical trials such as HIV vaccine trials may not need to consider season. Seasonal analyses are underway at three additional sites in Zambia, Uganda, and Kenya and may further inform these preliminary results.

P24 antigen screening for early detection of HIV infection in discordant heterosexual couples in Zambia

J Mulenga, E Hunter, O Manigart, G Stevens, S Allen and the Rwanda/Zambia HIV Research Group

Objective: Early detection of HIV infection is a critical endpoint in HIV prevention clinical trials and important in studies of HIV immunology and pathogenesis. Strategies to identify recent HIV infections include shorter, more frequent testing intervals and antigen detection methods.

Methods: Zambian couples discordant for HIV infection were enrolled through a Couples' Voluntary Counseling and Testing (CVCT) center and followed at 3-month intervals from July 2002 to December 2005. The HIV negative partner is serologically tested with rapid HIV tests (Abbott Determine® and Trinity Biotech Capillus®), and plasma is set aside for weekly p24 antigen (p24Ag) screening (Beckman-Coulter). HIV antibody-positive volunteers are counseled, and both partners are asked to provide additional samples of blood and genital fluids. HIV-antibody negative individuals who are p24Ag-positive are asked to return for repeat HIV-antibody testing and sample collection. Due to an elevated level of background the p24Ag positivity is defined as 5x the calculated cutoff of the test.

Results: The seroconversion rate in counseled HIV discordant Zambian couples is 7-8/100 person years. Of 148 seroconvertors identified in 42 months of the study, 32 (22%) were p24Ag+: Nine were HIV antibody-positive with two rapid tests at the time p24Ag was detected, and 23 were HIV antibody-negative. All 23 were HIV antibody-positive when they returned for repeat testing and sample collection (median 48 days), even though in some cases this was only 7-10 days later. Only 3 of the 23 were still p24Ag+ at re-draw (6-19 days later).

Conclusions: In a cohort with a seroconversion rate of approximately 2% per 3-month interval, almost one quarter of new infections can be identified at the p24Ag positive stage. The main obstacle to detection of recent infection is the short window of antigen positivity. Because increasing the frequency of study visits in this cohort may result in decreased retention, more frequent batching of p24Ag testing and same-day invitations for retesting are being employed to identify study participants in the earliest stages of infection.

Screening and Enrollment into a Phase I HIV Vaccine Trial in Kigali, Rwanda

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Background: A Phase I HIV Vaccine Trial, Protocol V001 required the enrollment of 64 (32 from both Rwanda and Kenya sites) healthy, HIV uninfected adults at low risk for HIV infection with a minimum of 30% female volunteers.

Objective: To present the experience of enrollment into IAVI's V001 study and the reasons for screening failure.

Methods: Concordant HIV negative couples were recruited from Couples' Voluntary Counseling and Testing Centers (CVCT). 45 couples had been followed for up to three years, others were referred directly from CVCT. Breastfeeding or pregnant women and those planning to conceive in the next year were screened out. Consenting was a three-stage process for both partners. First, Focus Group discussions on HIV vaccines were followed by group Education Sessions including the informed consent video. Couples were told that one member of the couple would be enrolled and priority would be given to the female. Finally, couples were invited for a second viewing of the consent video, a private question/answer session, Assessment of Understanding and consent signing. Medical and Laboratory screenings were completed to assess eligibility.

Results: 177 individuals (mostly partners) attended focus group sessions (90 women, 87 men), 115 volunteers signed consent forms and were screened (46 women, 69 men) and 35 volunteers were eligible of whom 32 were enrolled (12 women, 20 men). The reasons for screening failure were: clinically significant acute or chronic abnormality on history and/or physical exam (n=49), unable/unwilling to comply for the study period (n=10), eligible males whose wives were enrolled (n=7), positive HBsAg or anti-HCV antibody (n=5), high blood pressure (n=3), refusal of long-acting contraceptives (n=2), significant lab abnormalities (n=2), failure to demonstrate consent form understanding (n=1) and pregnancy (n=1).

Conclusion: We had targeted to enroll 50% women and expected that by involving the couple in the education and consenting process, we would remove potential barriers to female enrollment. 30% of men and women screened were eligible for enrollment (35/115). After pre-screening women for pregnancy, breastfeeding and desire to conceive and prioritizing women in couples where both partners were eligible, a substantial fraction of women (n=12, 38%) were enrolled.

Expansion and maintenance of an HIV discordant couple cohort in Kigali, Rwanda in preparation for vaccine efficacy trials

A Tichacek, E Kestelyn, E Karita, K Kayitesi, E Shutes, J Bizimana, E Chomba, P Fast, S Allen, and the Rwanda Zambia HIV Research Group

Objectives: The Global HIV/AIDS Vaccine Enterprise identified “expanding access to large, well-defined populations of uninfected people at high risk of HIV infection” as a gap in the clinical trials capacity of developing countries. With the majority of new HIV infections in Africa occurring within cohabiting couples, expanding cohorts of discordant couples to conduct HIV prevention trials is a priority.

Methods: Projet San Francisco in Kigali, Rwanda expanded couples’ voluntary counseling and testing (CVCT) in 2003 to recruit HIV discordant couples for prevention trials. Outreach is through word-of-mouth and invitation by trained outreach workers. After testing at one of three CVCT centers, discordant couples who have cohabited at least three months are invited to enroll in an open cohort. The HIV negative partner is followed quarterly, and couples receive reproductive outpatient care and ARV screening. Couples are recruited from the established cohort for vaccine and non-vaccine clinical trials.

Results: From 2003 to 2005, over 51,000 CVCT invitations were distributed by INAs (Influence Network Agents) and 25,522 couples were tested. Among those tested, 3176 (12%) couples were discordant (1329 male HIV+, 1847 female HIV+) and 1423 (45%) discordant couples were eligible for cohort enrollment. Most discordant couples not eligible either did not meet cohabitation requirements, or were determined not to be true couples. Among those eligible, 1190 (84%) enrolled. In December 2005 there were 1059 discordant couples in active follow-up, with retention >90% at 12 months. Since 2002, 50 seroconversions have been identified despite counseling (HIV incidence rate 3.6 cases/100py, 95% CI 2.6 to 6.8). In 2006, enrolled couples with the infected partner on ARV were released from follow-up to consolidate health care at the ARV clinics. Screening and enrollment for a Phase I HIV vaccine trial began in November 2005.

Conclusions: The establishment and retention of a well-defined, high-risk cohort in preparation for vaccine efficacy trials is possible in developing countries. Recruiting trial volunteers using a run-in design from an existing cohort with a known HIV incidence can improve volunteer follow-up and compliance. Eligibility criteria at CVCT and during cohort maintenance can be tailored to meet the needs of specific studies.

Recruiting HIV discordant couples for vaccine efficacy trials through Couples Voluntary Counseling and Testing centers in Lusaka, Zambia.

A Tichacek, C Vwalika, E Chomba, O Manigart, S Lakhi, M Krebs, P Fast, S Allen and the Rwanda Zambia HIV Research Group

Background: Cohabiting couples are the largest HIV risk group in Africa, and couples voluntary counseling and testing (CVCT) reduces transmission within couples by 60%. HIV discordant couples

retain relatively high rates of seroconversion despite counseling. HIV vaccines may reduce transmission further, and large cohorts of high risk individuals are needed for efficacy trials.

Methods: In Lusaka, cohorts of discordant couples have been recruited by the Zambia Emory HIV Research Project since 1995. Recruitment and testing efforts scaled up in 2002, when HIV vaccine and other clinical trials required expansion of discordant couple cohorts. Outreach is a mixture of word-of-mouth and invitation by trained outreach workers. After testing, interested discordant couples who have cohabited at least three months are enrolled for follow up. The HIV negative partner is followed quarterly, and couples receive reproductive outpatient care. Couples are recruited from established cohorts for HIV prevention trials, including vaccine trials.

Results: From 2002-2005, over 118,000 CVCT invitations were distributed by Influence Network Agents and 2082 discordant couples (909 with HIV+ men, 1173 with HIV+ women) were identified. Of those, 1620 (78%) met eligibility criteria for enrollment and 1130 (70%) of those were enrolled in the open cohort. In December 2005, 860 couples were in active follow up, including 208 couples enrolled before 2002. Retention of couples enrolled with follow up is 80% at 12 months. 147 seroconvertors (84 women and 63 men) have been identified (HIV incidence rate 8.3/100py, 95% CI 7.4 to 9.3). In 2006, participants on ARV were released from the cohort to consolidate health care at the district clinics and to focus cohort retention on couples eligible to participate in vaccine efficacy trials. Screening and enrollment for a Phase II HIV vaccine trial will begin in April 2006.

Conclusions: It is possible to recruit and retain sufficient numbers of HIV discordant couples for large scale clinical trials. This work will require substantial expansion of CVCT services, and the cohort maintenance run-in design ensures excellent retention in the clinical trial. Eligibility criteria at CVCT and during cohort maintenance can be tailored to meet the needs of specific clinical trials.

Gp41 HMA as a screening tool to study HIV transmission between partners

O Manigart, D Boeras, C Vwalika, P Ahues, B Chatora, J Mulenga, S Allen and E Hunter.

Background: The study of HIV heterosexual transmission is crucial to the design of better HIV vaccines. We have followed a cohort of discordant couples in Lusaka, Zambia for more than twelve years, in order to optimize prevention approaches to decrease transmission among couples. For those couples in whom transmission occurred despite three-monthly counseling and condom provision, the determination of epidemiologic linkage of viral strains between partners is critical for further virologic studies.

Objective: Development of a real time, rapid and easy to use tool to determine linkage of viral strains between partners.

Subject and Methods: The goal of this study was to determine the feasibility of using the heteroduplex mobility assay (HMA) to perform real time linkage analyses on transmission pairs. HMA was applied to 400 bp fragments PCR amplified from the PBMC DNA of each partner from 12 transmission pairs. After denaturation and renaturation of gp41 *env* DNA amplicons, hybridized DNA was electrophoresed on a polyacrylamide gel. A comparison of the HMA profiles from one individual (autologous) with the one obtained by mixing the gp41 PCR amplified DNA from both partners

(heterologous) was done. A slower migration of heterologous heteroduplexes in comparison with autologous ones was scored as an unlinked transmission as it depicts a diversity of more than 5% which is rarely found in gp41 sequences of linked transmissions. Results obtained were compared with direct sequencing.

Results: Twelve transmission pairs were randomly selected for PBMC DNA amplification and analysis by HMA and sequencing in the gp41 region. Among these couples, 4 were demonstrated as unlinked by gp41 HMA and 8 as linked. These results were confirmed by sequence analysis. In two cases, band heterogeneity was identified by HMA in the autologous samples, consistent with hypermutation observed in sequences of the same samples. Nevertheless, these samples were correctly identified as linked transmission events.

Conclusion: HMA in the gp41 region is an effective field-site method to study in real-time the epidemiologic linkage of HIV in transmission pairs.
