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TEN YEARS OF COMPLEMENTARY THERAPY USE AMONG PEOPLE WITH HIV/AIDS

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Plain Language Summary: Over the past decade there has been a series of advancements in medical treatments for HIV/AIDS. This project revisits a study that began in 1992 on approaches to complementary therapies by people with HIV/AIDS. In re-interviewing respondents involved in this initial study, we examined changes in the use of complementary therapies over time, especially in relation to the development of new medical treatments. This longitudinal approach makes it possible to identify both changes in the use of complementary therapies and the barriers that have continued to limit access to a range of health care options.

Objectives: This research revisits a study that began in 1992 on approaches to complementary therapies by people with HIV/AIDS. Respondents in this initial study had relatively few biomedical treatment options to draw upon. Over the past decade, however, there has been a series of advancements in medical treatments for HIV/AIDS. In re-interviewing respondents involved in this initial study, we examined changes in the use of complementary therapies over time, especially in relation to the development of new medical treatments.

Methods: We re-interviewed twelve respondents with diverse backgrounds who were involved in our initial research project. Our methodology followed an inductive and grounded approach. Interviews were semi structured and lasted approximately 1-2 hours. Respondents were asked to reflect on their approach to health care, with a particular focus on the use of complementary therapies, in relation to changes in their lives (such as health status) and to broad changes in the epidemic (such as developments in HIV/AIDS medications).

Results: Preliminary results identify several key points regarding the way PHAs have used complementary therapies over the last decade. First, respondents had sustained their commitment to complementary approaches to health even while having made greater use of medications in managing their HIV infection. Second, respondents noted that their use of complementary therapies had become a normal part of their everyday routine. This normalization was aided by the perception that health care professionals were more accepting of the role of complementary therapies in managing HIV/AIDS. Third, respondents expressed that very little had changed over the past decade in removing the barriers to accessing complementary therapies.

Conclusions: This research contributes to our understanding of health care decisions that people with HIV/AIDS make in relation to changing social context of the epidemic. This longitudinal approach makes it possible to identify both changes in the use of complementary therapies and the barriers that have continued to limit access to a range of health care options.

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COMPLEMENTARY THERAPIES FOR THE TREATMENT OF HIV: IN SEARCH OF THE EVIDENCE

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Plain Language Summary: The use of complementary and alternative therapies by people living with HIV/AIDS is widespread. However, to date there has been no systematic evaluation of the quality of evidence supporting the use of CAM for the treatment of HIV. We conducted a systematic review to locate all randomized trials of CAM for HIV and HIV related conditions. We found that cognitive behavioural interventions are supported by the evidence. However, despite their popularity, there are few trials to evaluate the effectiveness of herbal medicines, acupuncture or homeopathy.

Objectives: The use of complementary and alternative medicine (CAM) is widespread. Yet little is known about the evidence supporting its use in HIV/AIDS. We conducted a systematic review of randomized clinical trials assessing the effectiveness of complementary therapies for HIV and HIV related symptoms.

Methods: Comprehensive literature searches were performed of 7 electronic databases. Data was abstracted independently by two reviewers.

Results: Thirty trials met our predefined inclusion/exclusion criteria: 18 trials were of stress management; 5 of Natural Health Products; 4 of massage/therapeutic touch; 1 of acupuncture; and 2 of homeopathy. The trials were published between 1989 and 2003. Most trials were small and of limited methodological rigor. The results suggest that stress management may prove to be an effective way to increase the quality of life. For all other treatments, data are insufficient for demonstrating effectiveness.

Conclusions: Despite the widespread use of CAM by people living with HIV/AIDS, the effectiveness of these therapies has not been established. Vis a vis CAM's popularity, the paucity of clinical trials and their low methodological quality is concerning.

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NATURAL HEALTH PRODUCT-HIV DRUG INTERACTIONS: A SYSTEMATIC REVIEW OF CLINICAL TRIALS

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Plain Language Summary: The use of natural health products (NHPs) by people living with HIV/AIDS is widespread. We aimed to determine the number and quality of clinical trials examining drug interactions between these products and HIV drugs. We found 8 clinical trials and 1 population pharmacokinetics trials. Important interactions were observed with St. John's wort and garlic. Vitamin C may also interact but requires further study. Clearly, the issue drug interactions with HIV drugs is an important clinical issue and physicians should discuss NHP use with patients in an understanding manner.

Objectives: The use of natural health products (NHPs) within the HIV community is high. Several NHPs have demonstrated interactions with HIV medications that could contribute to drug failure. We aimed to determine the extent of clinical trials examining NHP-HIV drug interactions and the methodological characteristics, and results of the trials.

Methods: We searched 7 electronic databases and unpublished resources independently, in duplicate. We extracted information on NHP, HIV drug, study characteristics and effect sizes.

Results: Nine studies were identified, 8 clinical pharmacokinetics trials and 1 population-pharmacokinetics trials. Investigators studied 4 different herbal medicines (St. John's wort, Garlic, Goldenseal and Milk thistle) and one vitamin (Vitamin C). All 8 clinical trials investigated short dosing of PI's in steady state pharmacokinetics. All PI's used in these studies are metabolized primarily through the CYP 3A4 cytochrome. Significant interactions were observed in 1 trial of St. John's wort-indinavir and 1 population pharmacokinetics study of St. John's wort-nevirapine, 1 trial of garlic-saquinavir and 1 trial of Vitamin C-indinavir. Methodological challenges exist to making the results directly generalizable to patients.

Conclusions: Important drug level changes exist when NHPs are combined with HIV medications. Considering patient values and the implications of these studies, further research is urgently required to determine the extent of interactions with other commonly used NHPs.

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IMPACT OF AFRICAN HERBAL MEDICINES ON ANTIRETROVIRAL METABOLISM

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Plain Language Summary: We examined the effects of two African herbal medicines recommended by the South African Ministry of Health for HIV/AIDS patients on antiretroviral metabolism. We found that these herbal medicines had the potential to significantly alter antiretroviral metabolism and thus increase the potential for viral resistance and therapeutic failure.

Objectives: Recent policy developments in South Africa are implementing the provision of antiretroviral (ARV) drugs in combination with traditional African medicines. We determined the effects of 2 African herbal medicines recommended for use by HIV-infected patients on ARV metabolism.

Methods: Using established methods, we evaluated the effects of Hypoxis rooperii (African potato) and Sutherlandia on the CYP 3A4-mediated metabolism, P-Glycoprotein (Pgp) expression and Pregnane X receptor activity (PXR) in vitro.

Results: Both Hypoxis rooperii and Sutherlandia showed significant effects on CYP 3A4 activity, decreasing activity by 67.8% SD 16.5 and 87.1% SD 14.5 respectively, for the methanol extracts. As well, both Hypoxis and Sutherlandia induced the pregnane X receptor approximately 2-fold. Pgp protein was moderately inhibitory, with Hypoxis showing 42-51% and Sutherlandia showing 19-31% of the activity relative to 20 uM of verapamil.

Conclusions: These findings indicate that these herbs have the potential to substantially influence drug metabolism through multiple biotransformation pathways. Ingestion of these herbal products may lead to subtherapeutic levels of ARVs, and could increase the risk of resistance development and virological treatment failure.

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DEVELOPING AND VALIDATING A NEW HIV TREATMENT KNOWLEDGE TOOL (2004)

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Plain Language Summary: Patients often report feeling overwhelmed by the amount and complexity of the information they need to learn about their medical condition. This is especially true for complex medication conditions, like HIV, where there are many options and decisions to be made regarding the timing and type of treatment. Thus, the goal of this study was to develop a new scale to help HIV patients better understand and feel more knowledgeable about their HIV treatment care.

Objectives: How well patients understand their medical condition and treatment recommendations is one of the most salient predictors of treatment adherence across medical conditions (Dunbar-Jacob & Schlenk, 1996). This is equally true in the area of HIV where patients' baseline understanding of the importance of adherence and of the link between sub-optimal adherence and drug-resistance has been shown to predict adherence to HIV medications (Weiss et al., 2003). Although several HIV knowledge scales have been developed and validated, these measures typically focus on knowledge about HIV transmission, health behaviours, and medical facts and misconceptions about HIV. These scales do not specifically assess patients' understanding of more complex and specific HIV treatment issues (e.g., optimal adherence, HIV viral load suppression, drug resistance, and the necessity of multi-drug regimens). Thus, the present study aims to develop and validate a new HIV Treatment Knowledge Scale.

Methods: This scale is in the process of being developed and validated. Current items are based on an extensive literature review and in consultation with experts in the area of HIV care. A total of 12 items have been generated to date (e.g., "HIV is cured when the HIV viral load test results becomes 'undetectable'" and "If sexual partners are both HIV+, condoms are not needed because both partners already have HIV"). A pilot study was conducted in which this scale was administered to health care professionals (n=54).

Results: Results from the pilot study indicate that health care professionals demonstrated high levels of HIV treatment knowledge. The next phase is to generate more items and to administer the HIV treatment knowledge scale to various HIV patient populations.

Conclusions: The HIV Treatment Knowledge Scale is a novel measure that addresses important new HIV treatment-related issues which have not been assessed in existing HIV knowledge scales. Plans to further develop this scale are discussed, as are potential clinical uses of this knowledge tool. Observers are also invited to comment on scale items in an interactive forum.

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SUPPLEMENTAL TESTOSTERONE IN NONHYPOGONADAL MEN ON HAART MAY EXACERBATE VISCERAL ADIPOSITY AND CARDIOVASCULAR RISK

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Plain Language Summary: Supplemental testosterone (ST) has been associated with reductions of fat in deep tissues (the fat associated with risk of heart attacks) when used in middle aged men with low levels of testosterone and elevated belly fat. This type of fat is a common complication of HIV and its treatment and may be a contributor to future risk of heart attacks. We studied the use of ST in men on HAART to see if we could reduce belly fat, however failed to do so. The reasons for this are likely to be complex, however, the bottom line is that ST should be used with caution, particular among those who already had risk factors for heart disease.

Objectives: Lipodystrophy is a major treatment limiting side effect of antiretrovirals. This complication may be in the form of cosmetic subcutaneous fat lipoatrophy or, more concerning, visceral fat accumulation, which can contribute to cardiovascular risk. We undertook a study to determine if supplemental testosterone (ST) (used for indication of muscle wasting and improved quality of life (QOL)) had an effect on lipodystrophy.

Methods: Three groups were studied, all were male and on antiretrovirals, not on lipid agents or hypoglycemics. Group 1 was testosterone naïve, group 2 had been on ST long term and continued group 3 were new users of ST. We had baseline and follow up measures including the usual plus MRI to measure visceral adiposity (VAT). Initial dose of ST was 200 mg IM q month and increased to q 2 weeks after first 3 months of study, however not all participants took all doses.

Results: A total of 45 were enrolled and 29 had complete follow up data. Few 7% were hypogonadal, 51% had hypertriglyceridemia and 40% had visceral adiposity at baseline. Factors which were associated with VAT at baseline and follow up included age, caloric illness and serum testosterone. Factors which were associated with an increase in VAT over two time points were nonPi regimen and ST. Muscle mass fell among new users of ST.

Conclusions: The unexpected finding of increased VAT with ST may have resulted from not enrolling hypogonadal men, enrolling 60% without visceral adiposity and potentially underdosing of ST. This study suggests that use of ST for lipodystrophy should be undertaken with caution in persons with significant cardiovascular risk factors. As well, in the absence of resistance training, ST may have cosmetically undesirable effects.

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PATIENT SUPPORT AND EDUCATION FOR PROMOTING ADHERENCE TO HIGHLY ACTIVE ANTIRETROVIRAL THERAPY FOR HIV/AIDS

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Plain Language Summary: Strict adherence to prescribed regimens is required to derive maximal benefit from highly active antiretroviral treatment (HAART) in persons living with HIV/AIDS (PHAs). Strategies to improve adherence are therefore clearly important for persons taking antiretroviral medications. Some interventions, particularly those that involve multiple sessions and personalized counseling, may have a significant impact on improving adherence. Further research is required before any specific adherence improving strategy can confidently be incorporated into standard clinical practice.

Objectives: To update a systematic review of the research literature on the effectiveness of patient support strategies and education for improving adherence to HAART in PHAs.

Methods: A search of the following databases was performed from February 1999 to March 2004: MEDLINE, CINAHL, HEALTHSTAR, EMBASE, PSYCHINFO, Sociological Abstracts, Cochrane Central Registry, International Pharmaceutical Abstracts, Sociological Abstracts, and Science Citation Index. Controlled clinical trials and observational studies examining the effectiveness of support and education based interventions to improve adherence to HAART were considered for inclusion. Inclusion criteria included the presence of a comparison group and a measurement of adherence at a minimum of six weeks. Study selection, quality assessments and data abstraction were performed independently by two reviewers.

Results: Study heterogeneity with respect to differing populations, interventions, outcomes and follow-up did not allow for meta-analysis. We included 11 studies involving 1,464 people. All studies were randomized controlled trials published between 1999 and 2003. Sample sizes and duration ranged from 43 to 367 and from 12 to 72 weeks, respectively. Many of the interventions involved the provision of an educational component to improve adherence. These programs often addressed strategies for adherence, barriers to adherence, and the development of problem-solving skills. Eight of the eleven studies demonstrated a statistically significant advantage associated with the described adherence intervention. The studies had several methodological shortcomings. While the intervention arms were described in some detail, this was not the case for the control groups. It is therefore impossible to conclude that these groups were comparable. Treatment estimates may have been influenced by the variation of the types of support strategies offered, differences in follow-up times, and a lack of reporting on the method of allocation concealment. In addition, overestimation of treatment effects may result from the use of subjective outcome measures, a lack of control over the level of attention received between the experimental and control groups, and the lack of steps taken to avoid contamination and co-intervention.

Conclusions: Some interventions, particularly those that involve multiple sessions and personalized counseling, may have a significant impact on improving adherence. There is a need for standardization and methodological rigour in the conduct of adherence trials. Further research is required before any specific adherence improving strategy can confidently be incorporated into standard clinical practice.

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INTERNET-BASED POST-MARKETING SURVEILLANCE SYSTEM FOR PATIENTS WITH HIV/AIDS – PILOT PROJECT

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Plain Language Summary: This pilot project looked at the technical feasibility and the value of having a Website for PHAs to report on side effects to their anti-HIV drugs. A Website prototype was deployed from February to July 2004. All reports were verified by a health care professional. A total of 25 users reported 245 problems, 15 of which were side effects, and 3 were serious and/or unexpected. The Website stayed secure and stable. A more permanent Website should be implemented to see if patient reporting allow for an earlier detection of a pattern of side effects compared to health care professional reporting.

Objectives: To assess the technical feasibility and explore the potential value in implementing a large-scale Internet-based reporting system for patients with HIV/AIDS (PHAs).

Methods: A prototype was designed, tested with focus groups and individual interviews with PHAs, and deployed from February to July 2004. It consisted of a stand-alone secure Website for patients to report serious or unexpected side effects to their antiretrovirals. All reports were reviewed and validated by a health care professional that contacted reporters by phone.

Results: 245 reports were generated by 25 users, 18 of whom provided contact information. Only 154 reports from 15 reporters could be considered for analysis. 15 reports were side effects, 22 reports were adverse events and 115 reports were indeterminate. Causality to the drugs was correctly attributed by users in 11 reports. One side effect was unexpected and non serious, and two were expected and serious. The Website remained secure and stable throughout the pilot project. The average time per reporting session was approximately 40 minutes and the average time for generating one report was approximately 4 minutes, including the validation time.

Conclusions: This pilot project showed the technical feasibility of implementing a larger-scale trial and a high degree of interest from PHAs to participate in post-marketing surveillance. Three important side effects were reported, but with a high noise to signal ratio. This may in part be explained by the design of the Website, and in part by an inability of PHAs to differentiate adverse events from side effects and establish causality between drugs and an event. A larger scale and more permanent Website should be implemented to compare health care professional to patient reporting with regards to how early signals can be detected, and to determine ways to increase the signal to noise ratio in patient reporting.

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BARRIERS TO PARTICIPATING IN HIV DRUG TRIALS: A SYSTEMATIC REVIEW AND META-TRIANGULATION

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Plain Language Summary: Enroling participants in HIV drug trials is an important challenge. In order to identify important concerns and barriers, we conducted a systematic review of the qualitative literature and used an experimental meta-analysis technique to determine the extent to which the issues raised in qualitative studies are felt by the larger populations. We identified key concerns of fear of side effects; distrust of research or researchers, general concerns about research design; interference in everyday life or changes in routine; and social discrimination. This knowledge can assist trialists in enrolment and addressing patients concerns.

Objectives: Enrolling adequate participants into HIV experimental drug trials presents an important challenge. We aimed to systematically review the literature to identify barriers and concerns amongst HIV patients to participation in HIV clinical drug trials.

Methods: We undertook systematic searches of: AMED, Campbell Collaboration, CinAhl, Cochrane Library, Embase, ERIC, MedLine, and NHS EED, sought unpublished research through the National Research Register (UK) and Clinicaltrials.gov, and searched bibliographies of reviews. We included articles in any language using qualitative methods to address barriers or negative attitudes to participating in HIV drug trials. We independently reviewed studies for validity and content. For the purpose of triangulating the findings, we pooled the surveys that presented results as proportions as weighted data points and tested for heterogeneity and significance.

Results: We included 3 semi-structured interview studies, 2 open-ended questionnaires, and 9 quantitative studies. Major barriers to participation included fear of side effects; distrust of research or researchers, general concerns about research design; interference in everyday life or changes in routine; and social discrimination.

Conclusions: The findings of this study should aid drug trialists in developing strategies to maximize participation and co-operation in HIV clinical drug trials while adequately informing and protecting prospective participants.

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SCREENING FOR ANAL DYSPLASIA: CORRELATION OF HPV GENOTYPES AND E6 TRANSCRIPTS WITH ANAL PATHOLOGY

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Plain Language Summary: Cancer of the anus occurs at very high rates in HIV-positive gay men. We are conducting an anal cancer screening program: abnormal pre-cancerous areas can be seen and treated using high-resolution anoscopy. These cancers are caused by HPV. We found that certain HPV-associated proteins were more associated with pre-cancer. This will help detection.

Objectives: HIV-positive men with a history of anal-receptive intercourse are at high risk for anal dysplasia and anal cancer. The objective of this study is to determine whether HPV genotype and E6 transcripts are correlated with anal pathology in HIV-positive men with a history of anal-receptive intercourse.

Methods: As part of an anal cancer screening study, over 200 HIV-positive men with a history of anal- receptive intercourse have had anal cytology (Pap smears), HPV testing, and high resolution anoscopy with directed biopsy. HPV genotype was determined by a PCR/Line Blot assay (Roche Diagnostics). Viral load and E6 transcripts for HPV genotypes 16, 18, and 31 were determined, using type-specific real-time PCR assays performed in the LightCyclerTM.

Results: Dysplasia was noted on biopsy in 57% of subjects: high grade dysplasia (HSIL) in 23% and low-grade dysplasia (LSIL) in 36% of subjects. HPV was detected in 97% of subjects with multiple HPV types in 92%. Oncogenic HPV types were found in 88%: HPV 16 (39%), HPV 18 (24%), HPV 31(15%), HPV 53 (26%), HPV 52 (23%). The number of HPV genotypes present per biopsy was highly variable (1-13) and was higher for the LSIL and HSIL vs. the normal biopsies (P=0.02). The presence of E6 transcripts was different for HSIL, LSIL and Normal with respect to HPV 16 (P=0.048) but not HPV 18 (P=0.51) or HPV 31 (P=0.31). HPV 16 E6 transcripts were present more often for the HSIL vs. the normal biopsies (P=0.02). Total E6 transcript levels (combined HPV 16, 18, and 31) were significantly different for the normal, HSIL, and LSIL biopsies (P=0.047).

Conclusions: The number of HPV genotypes and the level of HPV E6 transcripts correlated with severity of anal pathology in HIV-positive men. These viral parameters should be investigated further as possible biomarkers for disease progression.

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THE EFFECT OF BETA-CAROTENE ON THE PHARMACOKINETICS OF NELFINAVIR AND M8

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Plain Language Summary: Beta-carotene supplements are used by PHAs for claimed benefits including immune modulation and as anti-oxidant. Previous studies suggest that beta-carotene may potentially cause clinically relevant interactions with the HIV protease inhibitors. This study investigated the effect of beta-carotene on the blood concentrations of nelfinavir and the metabolite M8. A preliminary analysis after 6 subjects suggests no clinically relevant effect of beta-carotene on the blood concentrations of nelfinavir or M8. However, final conclusions can only be drawn once the study is complete (target enrollment is 14 subjects).

Objectives: Beta-carotene (ß-Car) supplements are widely used for claimed benefits including immune modulation and as anti-oxidant. In-vitro studies suggest the potential for clinically relevant pharmacokinetic interactions between ß-Car and HIV protease inhibitors. However, in-vivo data are lacking. The current study investigated the effect of ß-Car on the steady-state pharmacokinetics (PK) of nelfinavir (NFV) and its equally active metabolite M8.

Methods: HIV-infected persons using NFV 1,250 mg twice-daily + at least 2 NRTIs were eligible for this study. 12h PK of NFV and M8 were assessed at baseline and after 4 weeks of co-administration of ß-Car 25,000 IU twice-daily, after ingestion of NFV with a standardized meal. NFV and M8 concentrations were measured with LC-MS/MS and noncompartmental PK analysis was used.

Results: Six patients (all men, mean age/weight 47.5 yrs/79.3kg) completed the study so far. Pharmaceutical analysis of the ß-Car formulation confirmed the content (mean 97.8% ß-Car) and stability of the product during the study period. Supplementation of ß-Car increased the plasma ß-Car concentration in all patients (median increase 62% (range 9.1-211%). The median (range) baseline AUC0-12h, Cmax and C12h of NFV was, respectively, 37.2 h*µg/mL (26.5-51.4), 5.4 µg/mL (3.80-6.85), and 1.23 µg/mL (0.76-4.75). Co-administration of ß-Car did not have a significant effect on these PK parameters (p>0.24). The geometric mean ratio (NFV+ß-Car to NFV) and 95% CI for the NFV AUC0-12h, Cmax and C12h was 0.97 (0.75-1.27), 0.99 (0.87-1.12), and 0.90 (0.43-1.88), respectively. Furthermore, the ratio of the AUC0-12h of M8 to NFV was not significantly affected by ß-Car supplementation (p=0.12). Co-administration of ß-Car was well tolerated. One patient reported occasional headaches and altered taste, possibly related to ß-Car intake.

Conclusions: These preliminary results suggest that supplementation of ß-Car at a dose of 25,000 IU twice-daily does not have a major impact on the plasma concentrations of NFV or M8. However, final conclusions can only be drawn once the study is complete (target enrollment is 14 subjects).

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FUNCTIONAL EXPRESSION OF ABCG2/BCRP PROTEIN IN BRAIN CAPILLARY ENDOTHELIAL CELLS: RELEVANCE TO THE TRANSPORT OF ANTIRETROVIRAL DRUGS

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Plain Language Summary: ABCG2 is a drug transporter that protects various organs in our body by removing potentially harmful drugs (anticancer, antiretrovirals) from cells. Some antiretrovirals have shown to interact with this transporter. Results show that ACBG2 is active in the cells that line the blood brain barrier in human and rat brain cells. Brain cells that have been treated with dexamethasone, a corticosteriod drug, show that ABCG2 expression is increased compared to untreated cells. The functionally active ABCG2 transporter may limit the brain permeability of various drugs, including anti-HIV drugs, from crossing the blood brain barrier.

Objectives: ABCG2/BCRP, a member of the ATP-binding cassette superfamily of transporters, is an energy-dependent, membrane-associated, efflux half-transporter. It confers multidrug resistance to various anticancer drugs such as mitoxantrone and doxorubicin, as well as the nucleoside analog, zidovudine, in HIV-1 infected CD4+ cells. Some protease inhibitors have shown to inhibit ABCG2-mediated transport. The objectives of this study are to examine the gene/protein expression and functional activity of ABCG2 in primary cultures of human brain endothelial cells (HBECs) and an immortalized rat brain endothelial cell line (RBE4). In addition, we investigate the inductive property of dexamethasone, a corticosteroid agent, on the protein expression and functional activity of ABCG2. Results from a clinical trial have suggested that anticancer drugs combined with dexamethasone for the treatment of AIDS-associated lymphoma/leukemia may be an effective regimen (Cortes et al., 2001).

Methods: Gene expression of ABCG2 in brain cell culture systems is measured by RT-PCR. Protein expression is determined by western blot analysis using a specific anti-BCRP antibody (BXP-21). The MCFMX100 human breast cancer cell line overexpressing ABCG2, along with the parent line (MCF-WT), were used as controls. Drug accumulation studies in the MCFMX100 and RBE4 cells employed ABCG2 radiolabeled substrate, 3H mitoxantrone (20nM), in the presence of ABCG2 specific inhibitors, fumitremorgin C (FTC) and its analog, ko143. For induction studies, RBE4 cells were treated for 3 days with 1uM dexamethasone.

Results: RT-PCR analysis detected ABCG2 mRNA expression in primary cultures of HBECs and in the RBE4 cell line. Western blot analysis shows protein expression in the predicted range of 70-72 kDa in both cell systems. Transport studies in the presence of specific inhibitors for ABCG2 (FTC, ko143), P-gp (PSC833) and MRP1 (MK571) demonstrate that ABCG2 is functionally active in the MCFMX100 cells. Mitoxantrone accumulation by RBE4 monolayer cells is significantly reduced in dexamethasone treated cells compared with controls. This reduction is abolished in the presence of ABCG2 specific inhibitors, FTC (5uM) and ko143 (1uM).
Conclusions: Our data suggest that ABCG2 may play a significant role in restricting the brain permeability of various pharmacological agents, including antiretrovirals, in brain microvessel endothelial cells and imply a possible cooperative role with other drug efflux transporters expressed at the blood-brain barrier i.e., P-glycoprotein and several multidrug resistance proteins.
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EXAMINING THE IMPACT OF COGNITIVE REHABILITATION ON HIV/AIDS-RELATED COGNITIVE SYMPTOMS USING FUNCTIONAL MAGNETIC RESONANCE IMAGING: A WORK IN PROGRESS

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Plain Language Summary: Up to 60% of people living with HIV report experiencing cognitive symptoms that make it difficult to carry out many everyday activities. We are using cutting-edge neuroimaging technology to examine how the affected brain regions of people living with HIV/AIDS respond to a cognitive rehabilitation program. Preliminary results from this study-in-progress will be presented. Specifically, differences in brain activation patterns between people with HIV infection and controls at baseline and after treatment will be discussed in relation to the hypothesis that the cognitive rehabilitation treatment will improve the overall efficiency of the brains of people with HIV.

Objectives: Our objectives are to use functional magnetic resonance imaging (fMRI) to compare the effect of HAART and cognitive rehabilitation on patterns of brain activation in people with HIV and examine how changes in patterns of brain activation relate to neuropsychological and functional outcomes. We predict that cognitive rehabilitation has a significant impact on brain functioning over and above the effects of HAART.

Methods: All participants received a neuropsychological assessment and completed questionnaires about functional skills. Participants on stable HAART were divided into two groups. One group received the cognitive rehabilitation and one served as a control-comparison group. Each participant completed an fMRI scan before and after the ten-week treatment period. During the scan, participants undertook a continuous performance task which activated brain regions associated with complex attention and working memory, two abilities commonly affected by HIV infection.

Results: Preliminary results indicate significant differences at the baseline scan between participants who are at different disease stages. Results at baseline replicate previous research, showing that key brain regions in people with HIV are overactivated relative to controls in order to compensate for inefficiency. Preliminary examination of the post scan results show subtle changes in the brain activation patterns that may be due to the effects of the cognitive rehabilitation treatment.

Conclusions: fMRI is an important technology for exploring the brain basis of the cognitive symptoms commonly associated with HIV infection. Clear differences in brain function can be seen between participants with HIV and seronegative controls. Although a work in progress, the preliminary results of this study suggest that fMRI is a more sensitive measure of neural change than neuropsychological testing. Results also suggest that cognitive rehabilitation is an effective means of treating cognitive symptoms. Future directions for the role of neuroimaging in understanding the basis of HIV-associated cognitive impairments will be discussed.

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IMMUNE AND ENDOCRINE DYSREGULATION IN HIV/AIDS: A POSSIBLE LINK WITH THE MANIFESTATION OF DEPRESSION, FATIGUE, AND NEUROCOGNITIVE SYMPTOMS?

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Plain Language Summary: We are trying to understand if the body's response to stress and infection with the HIV virus may be linked to changes in energy level, brain functioning, and/or mood. Our findings suggest that higher immune responses, as measured by signals found in the blood (IL-6 and neopterin), are associated with feeling down (depression) in some people with HIV/AIDS. This link may be useful for figuring out which people are at more risk of having longstanding depression when medications (antidepressants) may not work.

Objectives: This pilot study aimed to advance our current understanding of the role of biological processes in the development of specific neuropsychiatric complications in HIV/AIDS. In particular, this project explored whether elevated systemic indicators of immune system (i.e., IL-6, TNF-alpha, and neopterin) and hypothalamic-pituitary-adrenal axis (HPA) activation (i.e., cortisol) were associated with commonly reported clinical symptoms of cognitive impairment, fatigue, and depression.

Methods: Thirty-one HAART-naïve adults with HIV infection completed subjective symptom questionnaires and neuropsychological tests. Blood samples were obtained and processed at three separate laboratories. One-tailed Spearman correlations were used to examine the relations between serum concentrations of immune and HPA factors, cognitive impairment (learning efficiency, attention/working memory, and psychomotor processing speed), and subjective symptoms (fatigue, depression, cognitive complaints, and general illnesses).

Results: Neuropsychological impairment was not associated with the levels of biological markers. Depressive symptoms were, however, modestly associated with elevated IL-6 mRNA expression ($r_s = 0.40$, $p < .05$) even after removing the influences of fatigue, total cognitive complaints, and illness symptoms ($p_r = 0.39$, $p < .05$). Elevated serum neopterin was strongly associated with depressive symptoms in individuals taking antidepressants ($r_s = 0.83$, $p < .001$), though the association was nullified in the group not taking any antidepressants ($r_s = -0.25$, $p > .05$). More specifically, mean neopterin levels were higher in the depressed as compared with non-depressed group but only for those individuals taking antidepressants [$F(1,11) = 45.66$, $p < .001$].

Conclusions: Increased subjective symptoms, especially depression, may be associated with elevated immune activation (i.e., higher levels of IL-6 mRNA and neopterin) in some individuals with HIV infection. While antidepressants may exert immunosuppressive effects in treatment-responsive individuals, treatment-resistant individuals may continue to have both elevated depressive symptoms and neopterin levels despite presumably therapeutic doses on antidepressants. Although replication in a larger sample is needed, these preliminary findings suggest that systemic biological markers (especially neopterin) may be useful in differentiating individuals at greater risk of developing chronic, debilitating depression. Implementation of alternative interventions may be necessary to alleviate depressive symptoms in this selective group and ultimately improve their quality of life.

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PERFORMANCE OF A COMMERCIAL GENOTYPING SYSTEM FOR ANALYSIS OF DIVERSE HIV-1 STRAINS

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Plain Language Summary: The ViroSeq system can be used to analyze plasma samples with diverse HIV-1 strains. Availability of this genotyping system should facilitate studies of HIV-1 drug resistance in non-subtype B strains of HIV-1.

Objectives: The ViroSeqTM HIV-1 Genotyping System v2.0 is an FDA-cleared system for analysis of HIV-1 drug resistance mutations in subtype B strains. ViroSeq uses 9-10 oligonucleotide primers: 1 for reverse transcription, 2 for PCR amplification, and 6-7 for sequencing. Genetic differences among different subtypes and circulating recombinant forms (CRFs) could potentially influence assay performance. We evaluated ViroSeq performance for analysis of diverse HIV-1 strains.

Methods: Plasma samples were obtained from 126 individuals from Uganda, Cameroon, South Africa, Argentina, Brazil and Thailand. The samples were analyzed with ViroSeq in two laboratories. Pol region sequences obtained with ViroSeq were used for HIV-1 subtyping. Sequencing primer failures were analyzed by comparing sequencing primer and target sequences. Since the target sites for primers A and D are not within the ViroSeq processed sequence, raw sequence files from primer F were used for this analysis.

Results: PCR amplification was successful for 125 (99%) of the 126 samples and genotypes were obtained for 124 (98% of total). The subtype composition of the panel was: 16 A1/A2, 12 B, 13 C, 11 D, 9 F/F2, 7 G, 1 H, 9 CRF01_AE, 32 CRF02_AG, and 14 unique intersubtype recombinant strains. The identity of sequence data from the two laboratories ranged from 98% to 100% (median 99.8%). The overall success rate of the 6 primary sequencing primers was 95%. Overall, primer sites were well conserved. In most cases, the mean number of primer-template mismatches was 1 or less. The mean number of mismatches was slightly higher for primer A on subtype F and CRF02_AG, primer B on CRF01_AE, and primer G on subtype C. The least overall conservation was observed for primer H, with the highest number of mismatches for CRF02_AG strains, ranging from 0-5.

Conclusions: The ViroSeq system can be used to analyze plasma samples with diverse HIV-1 strains. Availability of this genotyping system should facilitate studies of HIV-1 drug resistance in non-subtype B strains of HIV-1.

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IMMUNOGENICITY OF SIMPLIFIED SIV IN CYNOMOLOGUS MACAQUES

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Plain Language Summary: We prepared variants of the monkey (simian) immunodeficiency virus, SIV, that were designed to replicate poorly in animals. These were administered as a vaccine to cynomologus macaques, both as free simplified virus and as a DNA genetic construct designed to produce the virus after administration to the animals. In no case did the animals become persistently infected, but immune responses to the virus were detected. We plan to readminister the vaccines in a final "boost" to enhance the immune responses, then to test their ability to resist a rectal challenge with unattenuated SIV.

Objectives: The scale of the HIV epidemic necessitates that all promising avenues for the development of a prophylactic vaccine should be explored. One of the approaches that has remained poorly investigated is the use of live, genetically attenuated, replication deficient viruses as vaccine agents. Here we seek to test the safety of genetically attenuated SIV clones in cynomologus macaques, as well as their ability to elicit antibody and T cell responses to the virus.

Methods: Two plasmid clones of SIVmac239 lacking accessory genes were constructed. 'Delta-6' lacked all genes except Gag, Pol, and Env, and 'Delta-5' included these genes and Rev. Virions were produced by transfection of these plasmids in Cos-7 cells, and these virions induced limited infection in CEMx174 and MT4 cells. Four monkeys per group were immunized with Delta-5 or Delta-6 viruses or saline IV and were boosted with the same viruses IV and IR. Subsequent boosts included immunization with Delta-5 or -6 plasmid or control.

Results: Animals were found negative by p27 ELISA, proviral PCR, and in vitro coculture for evidence of persistent replication of the immunizing virus. No evidence of reversion of the viruses to the virulent form was detected in long-term followup. Immune responses to the virus were seen by IFN-gamma ELISpot assay against a peptide library spanning the gag, pol, and env proteins in some immunized and no unimmunized animals. Antibody responses to the viral proteins were detected by western blot.

Conclusions: The results suggest that genetically attenuated SIV can be used safely as an immunogen without evidence of reversion to the virulent form. The immnized animals show evidence of T and B cell responses to the virus. The animals in this trial will receive a final virus and plasmid immunization and will be challenged using repeated low dose SIVmac239 administered rectally to assess the protective effect of this attenuated viral vaccine.

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IDENTIFICATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1)-SPECIFIC T HELPER LYMPHOCYTE EPITOPES

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Plain Language Summary: HIV poses the single greatest threat to humanity in our time. Designing strategies to combat its transmission is imperative to control the spread. CD4+ helper T (Th) cells are critical in the maintenance of anti-viral immunity and recognition of antigens in conjunction with class II HLA. HIV infection results in a preferential decline in the number of CD4+ cells. However, class II HLA variability has not been well applied to study HLA allele-specific immune responses following HIV infection. The present study focuses on investigating this little-explored area which could provide a model for the production of vaccines for HIV.

Objectives: Previous studies including that in our lab have shown the association of certain HLA types with disease progression (HLA-A*2301, A*29, B*22) or non-progression (B*14, C*8) and resistance to infection (HLA-A*2/*6802, B*58, DRB1). In this context, a comparative knowledge of the qualitative nature of epitope-specific Th response is required to identify those which are associated with better immune control of virus. Furthermore, despite the presence of well defined HLA class I restricted CD8 epitopes, the predictors on the maintenance of antigen-specific memory CD4+ T cells in HIV infection are not well understood. To address this issue, we sought to undertake a comprehensive analysis of the proliferative potential, breadth and magnitude of HLA-DRB1-*0101 and -*0102 directed Th responses in HIV infected individuals.

Methods: Participants were HLA typed using SSP-PCR. Peripheral blood mononuclear cells (PBMC) were prepared from apheresed blood. HIV gag-specific Th responses were evaluated on fresh PBMC using a panel of 15-mers spanning the entire HIV-1 (HXB2) gag with 11 amino acids overlap. HIV-1 gag was used owing to its established immunogenicity. CD4 responsiveness was assessed by interferon-gamma ELISPOTs, lymphoproliferative assay using 3H-thymidine and intracellular cytokine staining on CD8+ cell-depleted PBMC.

Results: HIV gag-specific CD4 responses were identified in majority of subjects and were found to be directed to epitopes over the entire protein. A variety of effector responses were detected including IL-2, IFN-gamma, TNF-alpha and cellular proliferation. Regions between gag 165-179 (SPEVIPMFSALSEGA) and 297-311 (VDRFYKTLRAEQASQ) were most immunodominant across the study group.

Conclusions: Multiple vigorous CD4 epitopes were identified and differences in the effector phenotype can now be correlated prospectively with HIV disease outcome to identify ideal CD4 epitopes for multi-epitope vaccine construction.

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EVALUATION OF VACCINE VECTORS IMPLEMENTED WITH IMMUNO-MODULATORY MECHANISMS AS HIV VACCINE CANDIDATES

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Plain Language Summary: HIV-specific cytotoxic T lymphocyte (CTL) responses play an important role in controlling viral replication and disease progression therefore the ability to elicit potent CTL has become a priority for AIDS vaccine candidates. However, induction of CTL memory responses of sufficient magnitude, breadth and durability remains a hurdle for CTL based vaccine to work effectively. We construct a number of DNA cassettes expressing the HIV genes in addition to the gene coding for an immune-modulator to regulate immune response to the vaccines. The immunogenicity of such vaccine candidates will be evaluated in BALB/C mice and the humanized A201 mice.

Objectives: HIV-specific CTL responses play an important role in controlling viral replication and disease progression therefore the ability to elicit potent CTL has become a priority for AIDS vaccine candidates. The virus-encoded immuno-modulatory proteins have drawn attention to their therapeutic potentials due to their act on the host defense system. One of the best-defined viral apoptosis inhibitors, M11L, has been shown to remain anti-apoptotic when expressed autonomously of any other myxoma protein. This provides the possibility of incorporating this apoptosis inhibitor into an HIV DNA vaccine to facilitate the persistence of the immunogen expression and to achieve better immunogenicity. In our study, we aim to demonstrate the ability of M11L-containing DNA vaccine vectors to resist apoptosis in vitro and in vivo and to evaluate the immunogenicity of an HIV DNA vaccine that co-expresses M11L in vivo.

Methods: Multiple DNA cassettes containing M11L have been constructed. In brief, they contain the CMV promoter, an intron and poly A signal, a CpG motif and the Rev independent, codon optimized HIV clade B envelope, Gag-pol or Nef sequences, in addition to the M11L gene. Similar gene expression cassettes are also introduced into a novel DNA vaccine vector pHERO that is stably maintained in dividing cells.

Results: Preliminary experiments showed the expression of HIV genes encoded by the cassettes in CEF cells and it will be compared with the expression level in the presence of M11L. Further in vivo experiments with these cassettes will be performed in BALB/C and the humanized A201 mice to evaluate the immunogenicity of these constructs.

Conclusions: Our study is aiming to address whether a vaccine can provide long-term protection against HIV. Positive results from this investigation will help overcome a major hurdle that limits the efficacy of current vaccine strategies and provide valuable insight into the correlates of protection against HIV infection.

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CTL RESPONSE IN DNA/MVA VACCINATED MACAQUES AND ITS INFLUENCE ON VIRAL QUASISPECIES DIVERSITY

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Plain Language Summary: DNA priming/Modified Vaccinia Ankara (MVA) boosting was used in our study to determine if cytotoxic T lymphocyte (CTL) response correlates with AIDS suppression. Four groups of macaques were studied. Blood was taken at multiple time points. CTL responses were assessed and virus was sequenced. Both an increased breadth and magnitude of CTL responses was seen among the animals receiving the higher dose of vaccine. In spite of mutations within some CTL epitopes, no generalized loss of immune control was identified out to 82 weeks post challenge, which is likely due to the broad CTL responses in these animals.

Objectives: To study the long term breadth and magnitude of CTL responses post vaccination/viral challenge and to determine if CTL diversification correlates with AIDS progression in macaques.

Methods: A vaccine regimen of DNA priming/Modified Vaccinia Ankara (MVA) boosting was used in a SHIV/rhesus macaque model. Four groups of a total 24 macaques were studied: Group 1 received high dose of vaccine (2.5mg) intradermally (i.d.); group 2 received high dose intramuscularly (i.m.); group 3 received low dose (250mg) i.d. and group 4 received low dose i.m. Blood was taken at multiple time points post challenge. PBMC were isolated and CTL responses were assessed with a set of 395 SIV Gag 9mer overlapping peptides using IFN-g ELISpot assay. MHC molecular typing was performed by PCR-SSP and the MHC restriction analysis of CTL epitopes was done by flow cytometry. RT-PCR was performed on viral RNA from plasma followed by TA cloning and sequencing to detect mutant variants in the pools of quasispecies in those animals.

Results: MHC type of all the animals was determined with PCR-SSP. CTL responses towards Gag peptides were detected over time in all the animals except one group 4 animal. Multiple new minimal CTL epitopes across Gag were identified. A trend towards both an increased breadth and magnitude of CTL responses was seen among the animals receiving the higher dose of vaccine. CTL responses were observed to expand and contract coincident with blips in viral load and shift to new epitopes coincident with development of viral mutations. Analysis of the ds/dn ratio of the viral sequences confirmed the strong immune selection pressure. In spite of mutations in some CTL epitopes, no generalized loss of immune control of viral load was identified out to 82 weeks post challenge.

Conclusions: A broad array of anti-SIV CTL responses can be detected up to 82 weeks post challenge. They are associated with persistent low viral load and lack of clinical disease progression. Viral mutations have been documented within CTL epitopes but no animal has developed an uncontrolled viral load over time (except for a group 4 animal with no CTL that died early post challenge). This is likely due to the broad specificity of multiple CTL responses. The maintenance of T helper responses was not assessed and may also play a role.

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IDENTIFICATION OF NEW HUMAN MHC CLASS II (HLA-DR)-RESTRICTED HIV-1 gag CD4+ T CELL EPITOPES BY OVERLAPPING PEPTIDE ELISPOT ANALYSIS OF gag DNA-IMMUNIZED HLA-DR TRANSGENIC/H2 CLASS II-DEFICIENT MICE

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Plain Language Summary: CD4 and CD8 T lymphocytes help eliminate most viral infections through recognition of specific viral peptides (epitopes) with MHC class II and class I molecules. For HIV-1, however, the high rate of viral replication, mutation, and immune selection limit the effectiveness of this T cell response, thus complicating full identification of HIV-1 epitopes. To avoid these factors for more complete epitope identification, we have used HIV-1 DNA vectors for genetic immunization of "humanized" mice expressing human MHC class II molecules. By identifying T cell epitopes under these conditions, we expect to find new targets for vaccine development.

Objectives: To identify HLA-DR1 and HLA-DR4-restricted HIV-1 gag CD4+ T cell epitopes in the absence of the influence of viral replication, mutation, and immune selection.

Methods: HLA transgenic (Tg) mice expressing the a and b chain genes for human MHC class II alleles HLA-DR1 or DR4 were bred onto a knock-out (KO) background deficient for mouse H2 class II expression (Tg HLA-DR1/ or -DR4/H2 class II-/- mice). Mice of each strain, along with non-Tg H2b wild-type and non-Tg/H2 class II-/- mice, were immunized with HIV-1 gag DNA vector by i.m. injection. Subsequently, splenocytes were used in ELISpot analyses with overlapping 15-mer peptides covering the full ~500 aa gag sequence (123 peptides total) (from the NIH AIDS Research/Reagent Program). Initial analyses used 23 pools of 5 adjacent peptides each, to localize the active regions within gag, while subsequent analyses tested individual peptides within each active pool.

Results: Compared to non-Tg class II-/- KO mice, which did not respond to any gag peptide or pool, Tg HLA-DR1/H2 class II--KO mice responded strongly to peptides 92 (p2p7p1p6 aa 2-16) and 108/109 (p2p7p1p6 aa 66-80/70-84), while Tg HLA-DR4 mice gave strong responses to peptides 10 (p17 aa 37-51) and 109 (p2p7p1p6 aa 70-84). HLA-DR-restricted epitopes have been reported in the vicinity of at least one of these peptides, indicating that our approach identifies both known and new epitopes. We also detected known peptide 36/37 (p24 aa 8-22/12-26) and new mouse I-Ab-restricted class II epitopes (p17 aa 73-87/77-91 and p24 aa 165-179/169-183) for non-Tg mice.

Conclusions: Analysis of gag DNA-immunized Tg HLA class II mouse T cells with overlapping full length peptides represents a novel approach for identifying new epitopes recognized in the context of human MHC class II molecules in the absence of the influence of infection. In addition to detecting known helper epitopes, we identified new epitopes with this approach. It will be important to test for recognition of these by T cells from HIV-1-infected humans.

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VIROLOGIC VS. IMMUNOLOGIC CONSERVATION PRESSURES ON IMMUNODOMINANT HIV CTL EPITOPES

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Plain Language Summary: This project focuses on immune cells, called cytotoxic T lymphocytes (CTLs), that recognize small fragments (epitopes) from proteins that make up the human immunodeficiency virus (HIV). Immunodominant epitopes are recognized by a particularly large number of CTLs within a single person, and by a large number of people within a population. They have been thought to be the best epitopes to include in HIV vaccines. Here we investigate an immunodominant epitope called SLYNTVATL (SL9), to test the idea that not all HIV epitopes evoke fully functional CTLs. Understanding which responses are most beneficial is crucial in designing HIV vaccines.

Objectives: We examine the virologic and immunologic pressures working to promote either conservation or variation in this epitope respectively. Specific issues include: whether the SL9 sequence is conserved due to virologic fitness and is therefore unmalleable by immunologic pressure, whether there are CTL driven mutations accumulating in this epitope in SL9-responsive infected patients, and whether the functional characteristics of the cells responding to the SL9 epitope correspond to fully functional, differentiated CTLs.

Methods: Mutations of the SL9 sequence are incorporated into the HIV plasmid clone pNL4-3. Viral viability is determined by monoinfection p24 assays and dual-infection competition assays are performed to determine the fitness of the mutant virus with respect to the wild-type virus. To test the mutation rate of the SL9 epitope in vivo, proviral DNA from HIV+ patient PBMCs is used in a quantitative limiting dilution PCR. The resulting amplicons are sequenced, and the epitope sequences in SL9-reactive and SL9-unreactive patients are compared. To assess the phenotype of SL9 reactive cells, the cytolytic function, cytokine secretion, and maturational status of SL9 tetramer positive cells is determined using FACS and ELISpot techniques.

Results: Plasmid constructs have been made to incorporate specific mutations at immunologically important SL9 residues (5,6 and 9) in Gag. Mono-infection fitness assays indicate that synonymous mutations in this region do not impact viral replication. Gag sequences from patients show several mutations throughout the epitope but so far no mutations in the C-terminal residue have been seen.

Conclusions: Mutations in the SL9 region of Gag do not appear to impair RNA trafficking during the lifecycle of HIV. However, an absolute conservation of the C-terminal MHC anchor residue of the epitope is seen in patient variant sequences. Therefore, future work on non-synonymous mutations may reveal viral fitness constraints that maintain a virologic conservational pressure on the epitope in vivo.

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CO-EXPRESSION OF MULTIPLE, VERSUS SINGLE, HUMAN MHC CLASS I ALLELES IN H2 DEFICIENT MICE INFLUENCES THE PATTERN OF VIRAL CTL EPITOPE RECOGNITION AND IMMUNODOMINANCE

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Plain Language Summary: After viral infection, our immune system uses different cells, including “killer” T cells, to fight off the infection. These “killer” T cells recognize and kill virally infected cells which display on their surface specific viral components ‘preferred’ by “killer” T cells. My goal is to identify and characterize these ‘preferred’ HIV-1 components in a “humanized” mouse model our lab has developed. To establish our approach, my initial studies have been performed with influenza A virus because much is known about this virus and it does not mutate within the host. The knowledge gained from these will aid in studies for HIV-1 and for vaccine development.

Objectives: To assess whether co-expression of multiple, as opposed to expression of single, MHC class I alleles influences the pattern of viral CTL epitope recognition and immunodominance.

Methods: A series of HLA Class I transgenic (Tg) mice were developed in our lab on a genetic background deficient for H2-K and H2-D class I expression. Single Tg HLA-B7/H2-Class I-/- and Tg HLA-B27/H2-Class I-/- mice were bred to generate doubly Tg HLA-B7/B27/H2-Class I-/- offspring, distinguished by (i) tail DNA PCR and (ii) flow cytometry with anti-HLA-B7 vs. anti-HLA-B27 specific mAb. Mice were infected with live influenza A (Flu A) virus and IFN-γ ELISpot assays were used to test for their specific CTL responses to known immunodominant B7-restricted (i.e. peptide NP418-426) or B27-restricted (i.e. peptide NP383-391) Flu epitopes. The H2-Db-restricted Flu epitope (i.e. NP366-374) was also tested for infected Non-Tg Wild Type and H2 Class I-/- mice.

Results: In response to influenza A infection, the CTL response in mice singly Tg for HLA-B27 or HLA-B7 is directed against the same viral peptides (i.e. NP383-391 and NP418-426, respectively) as recognized in HLA-B27+ and HLA-B7+ humans. Surprisingly, doubly transgenic mice (i.e. Tg HLA-B7/B27/H2-Class I-/-) showed a strong CTL response directed against the B7-restricted Flu epitope NP418-426, but a substantially decreased CTL response to B27-restricted Flu epitope NP383-391.

Conclusions: The results show that co-expression of HLA-B7 and HLA-B27 alters the pattern of viral epitope recognition by CTLs and immunodominance compared to infected mice expressing just a single HLA allele. Current studies will determine if this effect is due to both epitopes originating from the same viral protein. With respect to HIV-1, HLA-A2 is known to present gag77-85 while HLA-B27 presents gag263-272. Studies are underway in gag DNA immunized HLA-A2/B27 doubly Tg mice to determine if immunodominance of one response is also observed here. If so, vaccine strategies to overcome this effect should be considered.

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Immunology/Virology

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NEISSERIA GONORRHOEAE SUPPRESSION OF HIV-SPECIFIC IMMUNITY

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Plain Language Summary: Neisseria gonorrhoeae causes ~62 million new infections per year worldwide. Despite being a non-ulcerative sexually transmitted disease, gonorrhea enhances HIV-1 transmission by increasing both the shedding of virus by HIV-positive individuals and the susceptibility of seronegative individuals to HIV-1. Studies estimate that between 26% (USA)-44% (Africa) of new HIV infections could be averted if N. gonorrhoeae was eradicated. Remarkably, the molecular processes that mediate this relationship remain almost completely undescribed.

Objectives: We have recently demonstrated that N. gonorrhoeae Opa protein binding to human CEACAM1 suppresses CD4+ T cell activation and proliferation in response to various stimuli (Nature Immunol. 3:229-36). Given that most immune cell types express this receptor, and that >95% of gonococcal isolates bind CEACAM1, these results may explain why CD4+ T cell and CD8+ T cell counts decline and HIV loads increase during gonorrhea (Anzala et al. 2000; Kaul et al. 2002). Herein, we aim to ascertain whether the bacterial Opa proteins also suppress cell-mediated immune responses that are not involved in combating gonococcal infection but are critical for the clearance of HIV-1.

Methods: We have established in vitro infection protocols to allow definition of the effects of N. gonorrhoeae on (i) the maturation of human monocyte-derived dendritic cells (DCs) and (ii) cytotoxic T lymphocyte (CTL) activation in response to either heterologous (allotypic response) or autologous (HIV epitope-specific response) PBMCs. Immune cell responses to various activating stimuli in the presence of isogenic N. gonorrhoeae strains that either do or don't bind CEACAM1 were monitored by flow cytometric analyses of surface antigens and the detection of intracellular cytokines.

Results: N. gonorrhoeae variants that do not bind CEACAM1 stimulated both DC maturation and CTL activation. However, gonococcal expression of CEACAM1-specific Opa variants caused a significant reduction in CD8+ T cell activation and IFN-g expression. Opa-CEACAM1 binding also affected DC maturation, as evident both by the absence of surface CD83 and the DCs' reduced capacity to present allotypic and HIV-specific antigens.

Conclusions: Our studies demonstrate that gonococcal Opa protein binding to CEACAM1 has an incredible capacity to inhibit CD4+ and CD8+ T cell activation and DC maturation, each of which contribute to HIV-specific immunity. Our ongoing work aims to ascertain the combined effect of these activities on the DC and CTL-dependent killing of HIV-1-infected CD4+ T cells, with the ultimate goal of describing the molecular processes that mediate this dangerous synergy between these important human pathogens.

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IL-12, IL-23, IL-27 SECRETION AND CD83 EXPRESSION ON DENDRITIC CELLS AND THE EFFECTS OF HIV

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Plain Language Summary: Dendritic cells (DCs) are important in the initial response of the immune system to infection. Their role in HIV infection remains unclear. We hypothesize that HIV will affect DC maturation and function by downregulating cell surface markers and signaling molecules. This impaired maturation will prevent activation of the adaptive immune response and as such is a mechanism that prevents the successful clearance of HIV.

Objectives: Dendritic cells (DCs) are potent antigen presenting cells, and their role in eliciting immune responses in the context of HIV-1 infection remains unclear. We hypothesize that HIV-1 downregulates CD83, a well-characterized marker of maturation that has been implicated in T cell proliferation. This downregulation would be expected to result in less effective T cell proliferation and a lack immunological control of HIV-1, allowing for a more effective dissemination of the virus from infected DCs to T cells. With the anticipated block in maturation, we predict that IL-23 and IL-27 production would be downregulated, keeping memory T cells quiescent and downregulating IL-12 production, respectively.

Methods: DCs are isolated from skin obtained from patients undergoing paniculectomy. Langerhans cells (CD1a+) were obtained from the epidermal layer and dermal DCs (CD1c+) were obtained from the dermal layer. The layers were separated mechanically after overnight dispase digest, and cells obtained by microbead isolation after Ficoll-Hypaque gradient separation. Blood-derived DCs were isolated by CD1c+ microbeads. Monocyte-derived immature DCs (iDCs) were obtained after 6 day culture in the presence of IL-4 and GM-CSF. All cell types are analyzed by flow cytometry.

Results: Initial flow cytometry results show culturing monocytes for 48 hours prior to IL-4/GM-CSR is the optimal condition for generating iDCs. After 4 days in culture, the cells are positive for CD1c, CD1a, CD11C, low levels of CD80 and CD86, expressing MHCI and MHCII, CCR5 and low levels of CD1d. This phenotype correlates with an iDC phenotype. The loss of CD14 expression also supports the differentiation of monocytes into DCs. The cells obtained from microbead isolations (skin CD1a, CD1c, and blood CD1c) show a similar phenotype consistent with an iDCs phenotype when analyzed by flow cytometry. Analysis of IL-12, IL-23 and IL-27 production and CD83 expression is underway.

Conclusions: We have effectively isolated DCs from blood and skin (epidermal and dermal layers). Monocytes have been effectively cultured into a phenotype consistent with iDCs. The expression of CD83 and the production of IL-12, IL-23, IL-27 and the effect of HIV are being investigated.

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THE ROLE OF OX40-LIGATION TO ENHANCE HIV-1 SPECIFIC T CELL IMMUNITY

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Plain Language Summary: In viral infections that tend to persist, CD4+ T cells have been shown to play a crucial role in the maintenance of an effective ongoing CD8+ CTL responses. Deficient CD4+ T helper function in HIV-1 infection is responsible for the inability of CD8+ T cells to completely contain HIV-1 replication. It is currently unknown whether stimulating CD4+ T cells through OX40 ligand can further enhance CD4+ T cell help of CTL responses. The current study evaluated the role of OX40 signaling to enhance CD4+ T cell help of CTL responses against HIV-1 infections.

Objectives: Determine if CD4+ T cell help of virus specific CTL responses can be enhanced with OX40 receptor ligation

Methods: Ex vivo PBMCs obtained from HIV-1 infected individuals and HIV-1 negative EBV seropositive individuals were prepared in both unfractionated and CD4+ T cell-depleted conditions, and then treated with soluble recombinant human OX40 ligand-human IgG1 Fc fusion protein (hOX40L-IgG1); soluble human IgG1 protein was used as a control. Cells were cocultured with autologous monocyte-derived dendritic cells (MDDCs), either pulsed or non-pulsed with the specific HLA class I-restricted HIV-1 or EBV specific peptide. After 10-days of co-culture, CTL effector activity was assessed by intracellular staining for flow cytomeric analysis to enumerate the number of IFN-g-producing cells.

Results: OX40 is expressed constitutively on ex vivo human CD4+ T cells. HIV-1 infected individuals express higher OX40 on their CD4+ T cells (4.5%; range 1.8-7.5%; n=14) compared to HIV-1 uninfected individuals (1.3%; range 0.8-2.0%; n=6) ($p<0.05$). OX40 ligation of CD4+ T cells by human OX40L-IgG1 enhanced the ex vivo expansion of HIV-1 specific and EBV specific CTL from HIV-1 infected individuals and HIV-1 uninfected individuals, respectively. The most dramatically enhanced CTL responses were observed if CD4+ T cells were present. OX40 ligation did not enhance intracellular production of IL-2, IFN-g, or TNF-a in CD4+ T cells, not did it affect apoptosis of CD4+ T cells based on activated caspase-3 expression. OX40L stimulation enhanced CD4+ T cell proliferation by ~ 2% folds. Blocking IL-2 could not inhibit OX40L-induced CD4+ T cell proliferation nor OX40L-induced enhancement of HIV-1 specific CTL with soluble IL-2 receptor IL-2Ra.

Conclusions: OX40 ligation of CD4+ T cells by human OX40L-IgG1 enhanced the ex vivo expansion of HIV-1 specific and EBV specific CTL from HIV-1 infected individuals and HIV-1 uninfected individuals, respectively. The incorporation of OX40 ligand in the design of an anti-viral vaccine might enhance their ability to induce CTL immune responses in vivo.

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HUMAN IMMUNODEFICIENCY VIRUS-1 MODULATION OF CD8+ T-CELL CLONE GENE AND CXCR4 EXPRESSION

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Plain Language Summary: CD8+ T-cells have been shown to be targets of HIV infection. Since the factors required for HIV infection of CD8+ T-cells have been poorly studied, our objective was to examine the viral determinants required for entry of the virus in these cells. We found that HIV infection of the cells results in a modulation of numerous cellular genes and cell surface receptors. These factors may play a role in the increased infectivity and productivity of the virus in the CD8+ T-cell clones that were used in the experiments.

Objectives: The impact of HIV infection of CD8+ T-cells has been poorly studied. CD8+ T-cells have shown to be susceptible targets of HIV and during the course of infection, the activity and absolute numbers of these cells become hindered. Thus, we examined the mechanism(s) by which HIV enters CD8+ T-cells.

Methods: Primary CD8+ T-cells were first transformed by HTLV-I infection and later cloned by limiting dilution. Infection of the CD8+ T-cell clones with the HIV-IIIB strain was assessed by p24 ELISA and flow cytometry. Cellular expression of CD4, CD8 and the chemokine receptors (CXCR4 and CCR5) was performed by flow cytometry using fluorochrome-conjugated antibodies, whereas gene expression of these receptors was monitored by RT-PCR. Progeny HIV virus from infected CD8+ T-cell clones was sequenced to examine any mutations. In addition, microarray analysis was performed on one of the CD8+ T-cell clones. Lastly, expression of the HTLV-I tax gene in the clones was analyzed by nested PCR.

Results: From our results, we saw that the CD8+ T-cell clones can be infected at levels much greater than that of primary CD8+ T-cells. In addition, progeny virus from the clones did not yield any significant mutations. During the course of infection, there is no up-regulation or expression of CD4. However, the levels of CXCR4 expression decrease significantly from the surface of the CD8+ T-cell clones during in vitro infection. Similar results were obtained when examining the expression of the cell receptors at a gene expression level. Microarray analysis of the infected clones yielded a number of genes that are involved in HIV entry and exit to be up-regulated. Finally, we also found that the clones have integrated the HTLV-I genome but do not express HTLV-I viral gene products.

Conclusions: In sum, the CD8+ T-cell clones can be productively infected with HIV at greater levels than primary cells. This production was not dependent on CD4 expression or the process of HTLV-I transformation. Thus, viral entry into the clones may be facilitated through the use of chemokine receptors or other cellular receptors.

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DIFFERENTIAL REGULATION OF IL-7 RECEPTOR (CD127) ON CD8+ T CELLS: IMPLICATIONS FOR HIV IMMUNOPATHOGENESIS

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Plain Language Summary: Although not lost from the circulation, with disease progression cytotoxic T lymphocytes (CTL - also known as CD8 cells) become less able to contain numerous pathogens including HIV itself. Interleukin (IL)-7 and its signaling via the IL-7 receptor (CD127) is essential for optimal CTL activity. Our recent demonstration that significantly fewer CD8 cells from HIV(+) patients express CD127 as compared to healthy individuals, implicates a role for the regulation of CD127 expression in the immunopathogenesis of HIV, and we hypothesize that altered CD127 expression and/or function on CD8 cells contributes to impaired cell mediated immunity.

Objectives: IL-7 and its signaling via the IL-7 receptor (CD127) are required for normal CTL activity. Our previous observation that downregulation of this receptor is observed on CD8 cells of HIV infected individuals suggests that this may contribute to HIV-associated immunodeficiency. Initial studies indicated that TNFa and IL-7 decrease CD127 expression. Further elucidating the mechanism(s) of CD127 downregulation will provide additional insight into the immunopathogenesis of HIV disease.

Methods: Isolated CD8 cells from HIV(-) donors were incubated with TNFa or IL-7 for evaluation of CD127 surface expression by flow and RNA expression by PCR. Experiments with the inclusion of cyclohexamide or actinomycin D were carried out to determine the mechanism of TNFa induced CD127 regulation. Confocal microscopy and western blot analysis were performed to see if IL-7 induced receptor internalization of shedding. To determine the functional significance of TNFa mediated downregulation of CD127 on CD8 cells, response to IL-7 was evaluated in proliferation assays. Based on preliminary observations, since X4 and R5 strains of HIV appear to have differential effects on TNFa and IL-7 production, PBMCs from HIV(-) donors were incubated with a X4, R5, or a dual tropic clinical strain, with or without antibodies to TNFa, IL-7 or both, and CD127 expression on CD8 cells was analyzed by flow.

Results: TNFa induced a time and dose dependent decrease in the surface expression of CD127 and the accumulation of CD127 RNA. Cyclohexamide partially reversed the inhibitory effects of TNFa. Experiments with actinomycin D suggest that CD127 RNA degradation is enhanced by TNFa. IL-7 caused a transient decrease in CD127 surface expression whereas with higher concentrations this response was sustained. IL-7 has no effect on CD127 RNA levels or receptor internalization, but did induce shedding of CD127. Preincubation of CD8 cell with TNFa, and therefore decreased CD127 expression, resulted in blunted proliferative responses to IL-7.

Conclusions: IL-7R expression is downregulated by TNFa and IL-7, two cytokines that are upregulated during HIV infection. The mechanisms of action of these two cytokines appear to be distinct indicating a complex regulation of CD127 expression, and therefore IL-7 signaling, during HIV infection.

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COMBINED STRUCTURAL AND FUNCTIONAL APPROACH FOR IDENTIFYING NEW HUMAN MHC CLASS I-RESTRICTED VIRAL CTL EPITOPES IN VIVO

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Plain Language Summary: Finding an efficient HIV vaccine is a challenge. Like influenza, HIV can mutate and evade immune responses generated by vaccines. We believe that improved vaccines should also harvest the protection of "killer" T cells which current vaccines fail to do. "Killer" T cells limit the spread of infection by eliminating infected cells through recognizing viral components presented on their cell surface. Knowing that HLA genes are responsible for selecting specific viral components for presentation, we put the same genes into mice to uncover human-specific viral components that can be used with traditional vaccines for generation of concurrent "killer" responses.

Objectives: Identification of viral epitopes for stimulating CD8+ T cell responses when used as a vaccine.

Methods: We have generated a transgenic mouse (Tgm) model expressing HLA-B27 on an H2-Kb/H2-Db-double knockout background for identifying novel viral epitopes. By using influenza A as a validating model, we first studied whether an appropriate anti-viral response could be elicited in these mice as would be detected in B27+ humans. Using intracellular IFN-g staining and ELISPOT assays, we examined the anti-influenza response at the site of infection and in secondary lymphoid organs following primary and secondary infection. Influenza epitope candidates were predicted using two computer-based programs. These included NP.98-106, HA.200-208, M1.242-250, PB1.571-579, PB2.368-376, NS1.87-95 and PB2.702-710. The candidates were tested in influenza-infected HLA-B27 Tgm using ELISPOT assays.

Results: A significant proportion of the anti-influenza A CD8+ T cell response observed in HLA-B27 Tgm targeted the known B27-restricted NP.383-391 epitope. Kinetics of the overall anti-influenza response and viral peptide-specific response in the lung and spleen of HLA-B27 Tgm was comparable to non-Tg H2b wildtype mice, although the magnitude was less. Of the 7 peptide candidates tested, three (PB1.571-579, PB2.368-376 and PB2.702-710) were recognized in HLA-B27 Tgm during primary and secondary infection.

Conclusions: CD8+ T cells in HLA-B27 Tgm responded appropriately following influenza A challenge compared to non-Tg H2b wildtype mice. These CD8+ T cells recognized the known B27-restricted NP.383-391 peptide, suggesting that the antigen processing and presentation pathway is evolutionarily similar and able to support an analogous anti-influenza response in HLA-B27 Tgm as in B27+ humans. Of the 7 epitope candidates tested, PB1.571-579, PB2.368-376 and PB2.702-710 were recognized during primary and secondary infection. HIV-1 studies have begun in HLA-B27 Tgm. Following DNA immunization, B27-restricted gag.263-272-specific response was detected in these mice. Given the tendency of HIV-1 to escape CTLs specific for this epitope, it is important to identify additional conserved epitopes for development as possible T cell vaccines.

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HIV INFECTION OF PRIMARY MONOCYTES INHIBITS p38 AND JNK KINASES, LEADING TO DYSFUNCTIONAL NUCLEAR FACTOR BINDING AND DOWNREGULATION OF IL-12 p40 PROMOTER ACTIVITY

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Plain Language Summary: The immune response is composed of several systems of defense one of which is cell-mediated immunity (CMI). CMI is essential to pathogen control and clearance by the immune system, and is largely dependent upon IL-12. Progressive immunodeficiency in HIV infection is paralleled by a decrease in IL-12 production. Here we examine the cellular mechanisms by which HIV infection suppresses IL-12 expression.

Objectives: Cell-mediated immunity is essential to pathogen control and clearance by the immune system, and is largely dependent upon IL-12. Progressive immunodeficiency in HIV infection is paralleled by a decrease in IL-12 production. Here we examine the molecular mechanisms by which HIV infection suppresses IL-12 p40 expression.

Methods: Electromobility shift asay and supershifts were performed to determine if HIV influenced binding of transcription factors to the IL-12 p40 promoter. Luciferase assays using an adenovirus p40 promoter construct were performed in primary monocytes to assess the functional importance of sites within the p40 promoter. Western analyses were conducted to determine total and phosphorylated levels of the relevant upstream molecules MAP kinases (MAPK) (ie. p38, JNK and ERK) in HIV infected and uninfected monocytes. LPS stimulated monocytes in the presence and absence of MAPK inhibitors (SB203580 for p38, PD98059 for MEK/ERK1/2 and SP600125 for JNK) were investigated using both EMSA and luciferase assays.

Results: Increased nuclear factor binding to the NF-kB, AP-1, and Sp1 sites of the IL-12 p40 promoter was observed in HIV infected monocytes. By site-directed mutagenesis we determined that each of these sites was necessary for transcriptional activation of the IL-12 p40 promoter. Binding of NF-kB p50, c-Rel, IRF-1 and ICSBP was specifically impaired, while c-Fos and c-Jun binding to the AP-1 element was enhanced by HIV infection. Analysis of upstream MAP kinases demonstrated impaired phosphorylation of JNK and p38 MAPK. To determine if the effect of HIV infection on promoter function and IL-12 p40 production were due to the dysregulation in upstream kinases p38 and JNK, inhibitors of these kinases were used. MAPK inhibitors for p38 and JNK resulted in decreased IL-12p40 production. Use of these inhibitors lead to altered binding to the Sp-1, AP-1 and NF-kB sites. P38 and JNK inhibitors also resulted in decreased p40 promoter activity.

Conclusions: Decreased production of IL-12 observed in HIV infected monocytes appears to be a result of changes in inhibition of LPS induced p38 and JNK MAPK signalling. Inhibition MAPK function leads to dysfunctional nuclear factor binding to Sp-1, NF-kB and AP-1 as well as decreased p40 promoter activity and IL-12 production. Characterizing the intracellular mechanisms by which HIV inhibits IL-12 production will lead to a better understanding of how cell-mediated immune responses are inhibited by HIV infection and have the potential to lead to the development of novel immune based therapies.

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SEX HORMONES AFFECT LOCAL IMMUNE RESPONSES AND CORRELATE WITH PROTECTION FOLLOWING IMMUNIZATION IN GENITAL HERPES (HSV-2) INFECTIONS

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Plain Language Summary: The incidence of sexually transmitted infections (STIs) worldwide is rising despite increases in efforts and funds to research and treatment. It is known that HSV-2 infections enhance susceptibility to and increase transmission of HIV. One main strategy to control the spread of STIs is to develop effective vaccination regimes. Recent HSV-2 vaccine trials show limited success in a subgroup of female participants. This points to possible gender related factors, such as sex hormones estrogen(E2) and progesterone(P4). Sex hormones have been known to influence the immune system. Our own findings show that hormones can modulate the outcomes of HSV-2 infections.

Objectives: The present study was done to correlate the local and systemic immune responses to HSV-2 under the influence of sex hormones.

Methods: Female C57BL/6 mice were ovariectomized at 7 weeks of age and treated with the hormones estrogen and progesterone either singly or in combination. These mice were then immunized vaginally with an attenuated strain of HSV-2 (TK-) and challenged with wild-type virus 3 weeks later. HSV-2 specific activation in the iliac lymph nodes, spleen, and vaginal tract were examined through proliferation assays. Cell supernatants were collected and assayed for cytokines. Immune cells present in the genital tissue post HSV-2 infection were characterized with immunohistochemical localization and flow cytometry. Local IgA and IgG antibody titres were measured by ELISA.

Results: Viral titres indicated that the control (C), P4-treated, and 40% of E2+P4-treated mice were protected from HSV-2 challenge, while the E2-treated group was not. Further results showed that protection in immunized mice correlated with local immune responses. Increased lymphocytic proliferation in both the iliac lymph nodes(LN) and cells from vaginal tissues following HSV-2 specific stimulation was observed. Lymphoid aggregates consisting of CD11c+ cells and CD4+T cells were seen in the vaginal mucosa of protected groups. Further analysis by flow cytometry confirmed the increased numbers of CD4+T cells in protected P4 mice. Examination of cytokine profiles from LN supernatants of hormone-treated protected mice showed large increases in the amount of IFN-gamma and TNF-alpha. The protected E2+P4 group LN supernatants also had increased levels of IL-5 and IL-10. Increased local HSV-2 specific IgA and IgG production also correlated with protection.

Conclusions: These results show that protection from challenge following immunization may be determined by interactions of the local hormone environment with the induction of immune responses. This serves to emphasize the importance of hormone considerations in the design of successful vaccination regimes for women.

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NEISSERIA GONORRHOEAE STIMULATES HIV-1 EXPRESSION IN CD4+ LYMPHOCYTES

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Plain Language Summary: Gonorrhea is caused by the bacterium Neisseria gonorrhoeae. The occurrence of gonorrhea and HIV in the same individual is not uncommon, especially in developing countries. However, clinical evidence has suggested gonorrhea can have a negative impact on HIV infection by increasing viral loads, decreasing T cell counts and increasing the chance of transmitting HIV. How N. gonorrhoeae mediates these effects are unknown. We found that the bacteria can directly stimulate HIV-1 expression in HIV-infected T cells.

Objectives: Clinical evidence has shown that simultaneous infection with N. gonorrhoeae and HIV-1 increases both viremia and viral shedding into the genital tract, while also decreasing CD4+ T cell counts and CD8+ T cell responses. This study aims to decipher the molecular mechanism behind these observations by examining the effect of N. gonorrhoeae infection on HIV-1 expression in CD4+ lymphocytes.

Methods: Jurkat-derived 1G5 T cells, containing an integrated HIV-1 5'-LTR linked to luciferase, were exposed to various strains of N. gonorrhoeae. LTR-mediated transcription was measured by luciferase assay. Alternatively, primary CD4+ T lymphocytes and immortalized CD4+ T cell lines were either transfected with recombinant mutant proviral clones or infected in vitro with virus. Similarly, these cells were exposed to various recombinant strains of N. gonorrhoeae expressing defined virulence factors. HIV-1 expression was measured by FACS or ELISA for p24 antigen.

Results: N. gonorrhoeae was found to increase HIV-1 expression in both Jurkat 1G5 cells and cells containing full-length viral genome. Both methods demonstrated a dose-dependence relationship between the bacteria and HIV-1 expression over and above that seen with stimulation by PHA or PMA in cell lines. This affect is not caused by bacterial lipopolysaccharide, bacterial-derived DNA or formylated peptide, but is due, at least in part, to a proteinaceous component of the bacterium.

Conclusions: N. gonorrhoeae stimulates HIV-1 expression in CD4+ T cell lines. Ongoing efforts attempt to confirm this observation in primary cells as well as to decipher the exact bacterial product responsible for this effect.

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HIV SHEDDING IN SEMEN DOES NOT CORRELATE WITH SYSTEMIC CD8+ T CELL RESPONSES

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Plain Language Summary: Previous studies have shown that high numbers of virus-specific CD8 lymphocytes in the blood are associated with lower levels of HIV. Blood levels of HIV also correlate with how much virus is shed in semen, and exposure to infected semen is how many men and women acquire HIV. We studied whether numbers of virus-specific CD8 lymphocytes in blood correlated with levels of virus in either the blood or semen, but found no relation at all. In general, levels of semen virus mirrored levels of blood virus, but there several men shed much more (or less) virus in semen than expected.

Objectives: Most HIV transmission is sexual, resulting from direct contract with virus in infected genital tract secretions, and the amount of virus in semen often mirrors the amount in the blood. Blood virus-specific CD8+ T cell responses play a crucial role in control of HIV-1 levels in blood, and perhaps in semen. We decided to study this question.

Methods: Blood and semen samples were collected from 20 HIV-1 infected therapy-naïve men. Participants with leukocytes detected in first-void urine, or gonorrhea and/or chlamydia, were excluded. Blood and seminal viral loads were measured using the Versant HIV-1 RNA 3.0 Assay (bDNA). Blood HIV-specific CD8+ responses were evaluated using IFNg ELISPOT with a 15-mer peptide matrix (overlapping by 11) spanning the entire HIV-1 clade-B genome.

Results: Viral load in semen was ten-fold lower than in plasma (3.3 vs 4.3 log10; $P<0.001$). Viral loads in blood and semen were generally strongly correlated ($r=0.5$; $P=0.03$). However, in 15% (3/20) of men the semen viral load was comparable to that in blood, and semen virus was undetectable in 15% (3/20) men. Blood CD8+ T cells from participants recognized a mean of 8 HIV epitopes (range, 2-20 epitopes). These fell within Gag (37%), Pol (20%), Nef (20%), Env (13%), and less commonly within Tat, Rev, Vpr, Vpu or Vif. Neither the magnitude, breadth nor the specificity of blood CD8+ responses correlated with the viral load in either blood or semen.

Conclusions: Viral loads in blood and semen were closely correlated, although semen viral load was approximately ten-fold lower than blood. There was no association between blood HIV-specific CD8+ IFNg responses and levels of HIV RNA in either blood or semen. Future work will focus on identifying the immune correlates of HIV shedding in semen, which may be important in developing public health and therapeutic strategies to curb HIV-transmission.

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IMMUNE CORRELATES OF HIV SHEDDING IN SEMEN

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Plain Language Summary: The HIV pandemic is driven by the exposure of an uninfected person to genital secretions containing HIV during sex. However, how the immune system of an infected person controls the amount of virus in their genital secretions is not known. We have developed methods to measure various immune responses in the semen of HIV-infected men, including virus-specific CD8 lymphocytes. HIV-specific CD8 lymphocytes responses were present in the semen, but did not correlate with the amount of shedding – in fact, they were associated with higher levels of inflammation, which may indirectly increase semen shedding of virus.

Objectives: Sexual contact with HIV infected genital secretions, particularly semen, is the driving force behind the global HIV pandemic. However, little is known regarding the immune correlates of viral shedding in semen. We aimed to elucidate these immune correlates.

Methods: Blood and semen samples were collected from 27 HIV infected ART-naïve and 19 uninfected men. Participants with urethral inflammation or gonorrhea and/or chlamydia were excluded. Blood and semen viral loads were measured using the Versant HIV RNA 3.0 Assay (bDNA), and cytokine profiles were evaluated using a cytokine bead array (BD). Seminal HIV-specific CD8+ responses in blood and semen were assayed using IFNg intracellular cytokine staining, after short term ex vivo stimulation with a pool of 15mer peptide epitopes mapped in PBMC by ELISPOT.

Results: CD8+ T cells specific for epitopes mapped in blood were detected at a similar or higher frequencies in the semen, but neither the relative nor absolute number of seminal HIV-specific CD8+ T cells correlated with semen viral load. Increased levels of inflammatory cytokines (IL6, IL8, TNFa) in semen were positively correlated with the absolute number of seminal HIV-specific CD8+ T cells, and also with increased semen HIV shedding.

Conclusions: HIV-specific CD8+ T cell IFNg responses are present in high frequencies in semen. CD8+ IFNg responses are not directly associated with levels of semen virus shedding, but their association with inflammatory cytokines may indirectly lead to higher viral loads in the genital tract.

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MOLECULAR IMMUNE PHENOTYPING IN THE FEMALE GENITAL TRACT

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Plain Language Summary: Globally, women are at a higher risk for acquiring HIV from an infected partner than men. The female genital tract (FGT) is the site for acquisition and transmission of HIV. Technical limitations have hampered the study of FGT immune responses; sampling techniques yield variable cell numbers and traditional immune assays lack sensitivity. We have developed and validated a new molecular approach to measure the message levels of FGT immune genes that are important in HIV control. Our proposal will correlate the switching "on" or "off" of these immune genes with viral shedding and susceptibility in HIV-infected and uninfected women.

Objectives: The majority of sexual HIV transmission occurs across the mucosal genital membrane, and women are at a disproportionately higher risk for acquiring HIV from an infected partner. Innate and adaptive mucosal immune responses at the primary site of HIV infection in the female genital tract (FGT) are postulated to be important determinants of HIV transmission. An improved understanding of how perturbations in the FGT immune milieu might alter HIV transmission is crucial for designing mucosal HIV vaccines, but traditional assays of mucosal FGT responses have been hampered by small cell yields and poor sensitivity in measuring low frequency responses.

Methods: We developed a sensitive molecular approach to measure the steady state messenger RNA levels of innate and adaptive immune factors in the FGT. RNA was extracted from exfoliated cervical cells with a modified Trizol method. RNA was reverse transcribed to complementary DNA (cDNA) with random-nanomer primers and Sensiscript reverse transcriptase (Qiagen). Quantitative real-time polymerase chain reaction (QPCR) was performed with SYBR Green Technology (Stratagene) in 384-well format on the ABI7900HT Prism. Quantitative values, expressed as cycle threshold (Ct) were obtained during exponential amplification and relative expression levels were calculated with the “standard curve” method. Target gene expression is reported as average copy number (of triplicates) normalized to housekeeping gene expression.

Results: We generated the molecular profile of Toll-like receptors (TLRs) 1-10, DC-SIGN, stromal derived factor-1, IL2, IL4, IFNg, RANTES, MIP1a, MIP1b and HPRT (housekeeping gene) in 9 HIV-1 positive, 1 HIV negative and 3 HIV resistant women. In this small pilot study we observed very strong correlations between expression levels of certain TLR groups (TLR-1,3,4,6,8 in one cluster, and TLR-5,7,9 in another, $P<0.01$), as well as associations between distinct innate and acquired immune factors in the FGT (IL4 and TLR6, $P<0.05$; RANTES and TLR-1,3,4,8, $P<0.01$ for all).

Conclusions: We have developed a robust, reproducible assay to assess the expression of innate and adaptive immune factors in the FGT. Studies are underway to test this assay in HIV-infected and uninfected Toronto cohorts. We will correlate the molecular expression profile of innate and adaptive immune factors with flow cytometric measurements of endocervical immune cell populations, and HIV shedding patterns, and will extend our studies to define the influence of genital tract infections on the FGT immune milieu and HIV-1 shedding.

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EFFECTS OF Sam68 ON HIV-1 RNA PROCESSING

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Plain Language Summary: The HIV-1 Rev protein plays an essential role in the viral lifecycle in that it is required for production of the viral structural and enzymatic proteins. The correct processing of viral RNA is also a requirement for successful viral replication. Altering the state of the infected cell to make it unable to support either Rev function or viral RNA processing would inhibit progression of the viral lifecycle. I am working to determine the mechanism by which a human factor (Sam68) can regulate Rev activity and viral RNA processing.

Objectives: To determine the mechanism by which the host cell factor Sam68 enhances HIV-1 structural gene expression.

Methods: 293T cells were transfected with the appropriate plasmids and lysed for RNA or protein analysis. RNase protection assays were used to determine the distribution of 3' uncleaved and cleaved viral RNAs. Western blotting was used to analyze expression of the envelope glycoprotein gp120.

Results: HIV-1 Rev functions to export unspliced viral transcripts from the nucleus, allowing expression of the structural and enzymatic proteins of the virus. We have shown previously that Sam68 increases the proportion of unspliced, cleaved RNA, which is the substrate for Rev-mediated export. Here we show that the Sam68-like mammalian proteins (Slm1 and Slm2) also increase cleavage of unspliced viral RNA in the presence and absence of Rev. Interestingly, a deletion mutant of Sam68 (Sam97-255) containing only the GSG domain increases the proportion of unspliced, cleaved RNA only in the presence of Rev. These results suggest that Sam97-255 may be recruited to the RNA via the Rev protein while Sam68, Slm1 and Slm2 can bind the RNA in the absence of Rev. Further studies have shown that presence of the RRE is required for Sam97-255 stimulation of cleavage.

Conclusions: The fact that Slm1 and Slm2 can also stimulate cleavage of unspliced viral RNAs suggests a more general role of STAR-family proteins in the post-transcriptional regulation of HIV-1 RNA. Sam97-255 can enhance cleavage in the presence of Rev, suggesting the GSG domain contains all the sequences required for protein-protein interactions necessary for enhancement of cleavage.

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INVESTIGATION OF Sam68DC INHIBITION OF HIV-1 REPLICATION

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Plain Language Summary: The HIV-1 Rev protein plays an essential role in the viral lifecycle in that it is required for production of the viral structural and enzymatic proteins. Altering the state of the infected cell to make it unable to support Rev function would inhibit progression of the viral lifecycle. A deletion of the human protein Sam68, Sam68DC, is able to specifically inhibit the expression of viral proteins controlled by Rev. I am working to determine the mechanism by which Sam68DC can inhibit Rev activity.

Objectives: To determine the mechanism by which the deletion of Sam68, Sam68DC, inhibits HIV-1 structural gene expression.

Methods: All experiments were carried out in cell culture, and transfections were used to introduce viral genes and Sam68DC. A variety of microbiology techniques were used to analyze the effects of Sam68DC on the viral genes including: RNA in situ hybridization, immunofluorescence, immunoprecipitation, Western blots, polysome gradients and RT-PCR.

Results: Sam68DC inhibits the translation of Rev-responsive viral RNA by bundling the RNA around the periphery of the nucleus. Previously, I showed that disruption of the microfilaments, but not the microtubules, dissolves the Sam68DC-RNA structures. In this study I show that even once the viral RNA is diffuse in the cytoplasm it is not translationally competent. This indicates that either Sam68DC is still contacting the RNA and preventing access by the ribosome or that Sam68DC is altering the RNA to make it translationally incompetent. I have used polysome gradients to look at the postion of the viral RNA, and also analyzed the status of the RNA.

Conclusions: Sam68DC bundling of Rev-responsive viral RNAs does not cause inhibition of translation. Sam68DC works through an alternate mechanism to render the target RNA translationally incompetent, even when it is diffuse in the cytoplasm.

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TARGETING HIV-1 PROVIRAL DNA BY MODIFIED GROUP II INTRONS

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Plain Language Summary: Group II introns are able to splice out and insert themselves into their target site. By some modifications, the introns will be able to insert themselves into specific sites in HIV-1 proviral DNA. We also did some more modifications, so that this insertion leads to inactivation of HIV-1 DNA. We could show that the insertion happens. Now we are trying to test the insertional inactivation in inhibition of HIV replication.

Objectives: As a new approach to HIV-1gene therapy, we try to target HIV-1 provirus DNA with a modified group II intron. The advantage of this strategy over others, which target the HIV-1 RNA/proteins, is that it may confer a complete and permanent inhibition of HIV-1 replication in an infected cell.

Methods: Three group II introns have been modified by and cloning a designed cassette into the domain IV of the introns. The cassette contains a neo gene, a multiple cloning site, and a terminator. It was cloned in antisense orientation into the pACD-54a (a vector expressing the intron that targets the antisense strand at nucleotide 54). After removing the terminator, the cassette was also cloned in sense orientation into pACD-4021s and pACD-4069s (expressing the introns targeting the antisense strands).

Results: E. coli cells were co-transformed with both modified introns and recipient vectors containing fragments of HIV-1 DNA, and it has been shown that the modification of intron did not alter its mobility properties.

Conclusions: The intron-inserted pNL4-3 will be used for producing HIV-1 viruses. If the HIV-1 DNA is inactivated, studies will be performed to determine the effectiveness and frequency of insertional inactivation in mammalian cells. Next, E. coli cells will be co-transformed by modified introns and pNL4-3 (a vector containing the whole HIV-1 genome) to test the insertion of modified intron to the entire sequence of HIV-1 genome.

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INHIBITION OF HIV INFECTION BY THE NOVEL SOLUBLE MIMICS OF gp120 BINDING GLYCOLIPIDS: UNDERSTANDING THE MECHANISM

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Plain Language Summary: Glyscosphingolipids (GSL) are lipid (fat)-sugar conjugates within the cell membrane, that are required for HIV infection. We have made soluble derivatives of several GSLs that are able to bind to an outer surface HIV protein called gp120. The modified GSL globotriaosylceramide, called adamantylGb3, inhibits HIV fusion and infection in the test tube, irrespective of the type of HIV. Other modified GSLs, adamantyl galactosyl ceramide and adamantyl sulfogalactosyl ceramide, inhibit HIV infection but not fusion, and are also able to kill cells. AdamantylGb3 is more promising as a basis for a new approach to treatment of HIV/AIDS.

Objectives: Glycosphingolipids (GSLs) have long been implicated in HIV infection, and several bind to HIV gp120. We have shown that a water-soluble analogue of the glycolipid, globotriaosyl ceramide (Gb3), is a highly effective ligand for gp120 in vitro. This analogue, adamantylGb3, also inhibits X4 HIV-1 infection of a T-cell line and PHA/IL-2 activated peripheral blood-derived mononuclear cells (PBMCs) in vitro. We have also shown other GSL analogues with gp120 binding affinity; namely adamantyl sulfogalactosyl ceramide (SGC) and adamantyl galactosyl ceramide (GC) inhibit X4 HIV-1 infection of a T-cell line in vitro, and, with the exception of adamantylGC, prevent infection of PHA/IL-2 activated PBMC. We have now investigated the mechanism behind inhibitory effects of adamantylGSL analogues.

Methods: To confirm a broadly applicable inhibition, R5 HIV-1Ba-L was pre-incubated with increasing doses of adamantylGSL analogues, and PHA-activated PBMCs were infected with pre-treated HIV-1Ba-L. To ascertain whether inhibition was due to interference with membrane fusion, HeLa cells expressing X4 or R5 HIV-1 gp120 were pre-incubated with adamantylGSLs, and co-cultured with NIH3T3 fibroblasts expressing CD4 and CXCR4 or CCR5 respectively. Potential cytotoxic effects were determined by pre-incubating Jurkat T-cells and PHA or PHA/IL-2 activated PBMCs with adamantylGSLs. Cell death was measured by staining with AnnexinV-FITC and PI, and analysed by flow cytometry.

Results: HIV-1Ba-L infection of PBMCs was inhibited by adamantylGb3 with an IC50 of ~150μM. AdamantylSGC and adamantylGC inhibited HIV-1Ba-L infection with a similar dose response. In the presence of adamantylGb3, both X4 and R5 HIV-1 envelopes failed to fuse with respective NIH3T3 targets. However, membrane fusion was not blocked by adamantylSGC or adamantylGC treatment. Cell death in adamantylGb3 treated Jurkat T-cells, and PHA or PHA/IL-2 activated PBMCs was less than 10% compared to controls. In contrast, cell death associated with adamantylSGC and adamantylGC was significantly higher. In comparison, adamantylGC was more cytotoxic for PHA activated PBMCs, adamantylSGC appeared to be slightly more cytotoxic for Jurkat T-cells, while both were highly cytotoxic for PHA/IL-2 activated PBMCs.

Conclusions: AdamantylGb3 is a powerful inhibitor of X4 and R5 HIV-1 infection in vitro, interfering with HIV-host cell membrane fusion. This inhibition does not appear to be attributed to cytotoxic effects. AdamantylSGC and adamantylGC are inhibitors of X4 and R5 HIV-1 infection in vitro (with the exception previously stated). However, inhibition is not due to interference with membrane fusion, suggesting an alternative mechanism, potentially direct cytotoxicity. In conclusion, adamantylGb3 holds the most promise for future therapeutic strategies.

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MODULATION OF DRUG EFFLUX TRANSPORTERS IN GLIAL CELLS TREATED WITH THE HIV-1 VIRAL COAT PROTEIN gp120

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Plain Language Summary: Treatment of brain HIV-1 infection is difficult because anti-HIV drugs do not reach appropriate levels, which may be due to the presence of drug export pumps in brain cells that are infected by the virus. Our laboratory has shown that these pumps transport many antiretroviral drugs. The goal of this project is to determine if the HIV-1 protein gp120 can affect the presence and/or activity of drug export pumps. Results from our laboratory have shown that the level of drug export pumps may change in brain cells treated with gp120, suggesting that HIV-1 infection may affect the amount of anti-HIV drugs that reaches the brain.

Objectives: Human immunodeficiency virus type 1 (HIV-1) infection of the central nervous system (CNS) may result in HIV-1 encephalitis (HIVE), a chronic neurodegenerative condition (Kaul et al. 2001). The pathological events associated with HIVE (i.e., production/secretion of cytokines and nitric oxide) may be modelled in vitro by the exposure of cultured glial cells to the HIV-1 viral coat protein gp120. Chemokine receptors (i.e., CXCR4, CCR5) expressed at the surface of glial cells are known to mediate gp120 response (Persidsky & Gendelman, 2003). An obstacle to the pharmacological treatment of HIVE may be the expression of ATP-dependent, membrane-bound efflux drug transporters (i.e., P-gp, Mrp1) in brain cellular targets of HIV-1 infection (i.e., astrocytes, microglia). We have recently confirmed the functional expression of P-gp and Mrp1 in astrocytes and microglia and their role in the active export of anti-HIV drugs from several brain cellular compartments (Dallas et al. 2003, 2004; Ronaldson et al. 2004a, 2004b). However, it is unknown if in vitro HIV-1 infection of glial cells and/or cells triggered with HIV-1 viral proteins can alter the molecular expression and functional activity of these efflux drug transporters. The goal of this project is to investigate the effect of gp120 treatment on P-gp and Mrp1 expression in primary cultures of glial and brain microvessel endothelial cells.

Methods: Primary cultures of rat and human astrocytes, rat microglia, and human brain endothelial cells were used for this study. Primary cultures of rat astrocytes were incubated for the desired time (i.e., 6 h, 12 h, 24 h) in the presence of 0.5 nM gp120. Gene and protein expression were determined by RT-PCR and immunoblotting analysis respectively.

Results: CXCR4 and CCR5 mRNA were detected in all cell culture systems examined. The expression of CXCR4 and CCR5 proteins were confirmed in primary cultures of rat astrocytes and microglia. Semiquantitative RT-PCR analysis demonstrated increased expression of TNF-α, IL-1β, IL-6, and inducible nitric oxide synthase mRNA (relative to the appropriate housekeeping gene) in rat astrocyte cultures treated with 0.5 nM gp120. Following 6 h and 24 h exposure with gp120, P-gp protein expression was decreased in rat astrocyte cultures while the expression of Mrp1 protein was increased.

Conclusions: Gp120 treatment of glial cells may alter the expression of P-gp and Mrp1 in primary cultures of rat astrocytes suggesting that complex drug-transporter interactions may occur in the pathological response associated with HIVE. Supported by the Ontario HIV Treatment Network, Ontario Ministry of Health, and the Canadian Institutes of Health Research.

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VIRCO®TYPE ANALYSIS OF AN HIV-1 GENOTYPE PROVIDES A HIGHLY ACCURATE PREDICTION OF DRUG SUSCEPTIBILITY, COMPARABLE TO MULTIPLE REPEAT PHENOTYPE MEASUREMENTS

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Objectives: Virco®TYPE predicts antiretroviral drug resistance from viral genotypes through selection of isolates with similar patterns of resistance-associated mutations from a database (>32,000 clinical isolates). Their phenotypes are averaged to predict fold-change (FC) in IC50 relative to a reference wild-type. We calculated the accuracy of the predicted FC and assessed the contribution of phenotype assay variability to observed differences between predicted and measured FC.

Methods: Drug susceptibility was predicted for a panel of 7 clinical isolates with a broad range of drug resistance levels. The isolates were phenotyped 4 - 11 times over a period of 2 years against 16 drugs, comprising a total of 733 measurements. An analysis of variance was performed. FC, mean FC, and 95% confidence intervals were calculated for each drug for 1) individual phenotype tests, 2) the mean of replicate phenotype tests, and 3) predicted values.

Results: Root mean square error (RMSE), an estimator of the standard deviation of the difference between measured and predicted phenotype, averaged 0.35 logFC (range: 0.27, lamivudine to 0.6, delavirdine). Phenotype assay variability was the major contributor to differences between measured and predicted FC, accounting, on average, for 81% of RMSE. Predicted FC corresponded closely ($r^2=0.93$, $p<<0.0001$) to the mean of replicate phenotypes. For this set of isolates, numbers of matching geno-phenotypes ranged from 25 to 14318 (median = 325). Consequently, the confidence intervals for predicted FC were very small compared to those for individual or replicate phenotypes.

Conclusions: Virco®TYPE analysis of HIV-1 genotype provides a highly accurate prediction of drug susceptibility phenotype. By averaging results across multiple isolates with similar mutational patterns, this method overcomes the variability inherent to any biological assay.

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Global Impact of HIV

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THE CANADA AIDS RUSSIA PROJECT (CARP): A SUCCESSFUL MULTI-DISCIPLINARY MODEL

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Plain Language Summary: HIV has been increasing in Russia at a faster rate than in any other country. A Government of Canada-sponsored program aims to enhance the Russian capacity to deal with HIV. This multi-disciplinary program has taught protocol development, good clinical practice, delivery and effective utilization of antiretroviral therapy, enhanced the capacity of the laboratories and helped to develop strategies to reduce new infections.

Objectives: HIV has been increasing in Russia at a faster rate than in any other country. This project aims to enhance the capacity of governmental and non-governmental organizations (NGO's) to respond more effectively to the spread of HIV.

Methods: The project is funded by the Canadian International Development Agency and is based on a partnership between CARP and three of Russia's leading HIV/AIDS institutions: the Russian Federal Scientific-Methodological Centre for the Fight Against AIDS, the Russian Ministry of Health's Department of HIV/AIDS and AIDS Infoshare (a leading HIV/AIDS NGO). It also includes four regional AIDS Centres and their NGO's in Altai and Krasnoyarsk (Siberia); and Saratov and Tver. The aims are to enhance the capacity of key Russian organizations and to establish an integrated multidisciplinary network.

Results: Specific project activities have included: (1) A training component to ensure skills and knowledge transfer (2) Enhancement of information systems (3) Training of Russian specialists in Canada (4) Demonstration projects in adult and pediatric clinical care; prevention of maternal to child transmission; and laboratory testing (5) Involvement and training of community-based organizations (6) Engagement of Russian policy makers and (7) an enhanced focus on social-behavioural perspectives aimed at prevention. The project has taught protocol development, guidelines for good clinical practice, facilitated the delivery and effective utilization of antiretroviral therapy, enhanced the capacity of the laboratories and helped to develop strategies to reduce new infections.

Conclusions: As the HIV epidemic matures and spreads in Russia, there will have to be enhanced behavioural surveillance and prevention programs, delivery of cheaper antiretrovirals, and improved capacity to care for increasingly sicker patients.

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RISK FACTORS FOR HIV-1 TRANSMISSION IN AFRICA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Plain Language Summary: In Africa, infection with the human immunodeficiency virus (HIV) within the adult population occurs mainly through heterosexual intercourse. There is an urgent need to better quantify HIV-1 transmission in Africa, including estimates of HIV-1 risk factors in high-risk populations. This meta-analysis was performed to pool together the current published literature on risk factors for HIV-1 infection in Africa to quantify each risk factor. We hope to widen the current understanding of the factors that lead to HIV-1 acquisition. Such understanding is key to better control programs, in terms of coverage of effective interventions and evaluating the success of interventions.

Objectives: To systematically review studies to quantify and determine the most important risk factors for HIV-1 infection in Africa. There have been virtually no studies summarizing these findings in a meta-analysis.

Methods: We aimed to identify all studies published up to the end of August 2004 of risk factors for HIV-1 infection in Africa. Medline, Premed line databases were searched for papers that included 'Africa', 'HIV' and 'risk factor' as keywords or text in the abstract. This search yielded 1204 hits. We found 788 abstracts that met the eligibility criteria. Based on a single reading of all abstracts, 492 articles were obtained and these were read independently by two research staff to decide inclusion into the study based on a checklist for reporting meta-analyses of observational studies. A wider search strategy was then implemented with 'HIV,' 'HIV-1,' or 'delta retrovirus,' 'horizontal transmission,' or 'risk factor' and 'Africa'. Medline, Embase, Web of Science were searched using appropriate text words. Searches were also undertaken by hand with reference lists from these papers, and review articles.

Results: All major risk factors for HIV-1 infection will be quantified. 177 papers with relevant content were retrieved. Independent reviewers selected studies, assessed study quality and extracted data. We stratified studies based on study design and on whether they included participants from the general population or high-risk groups (such as patients treated for sexually transmitted infections). The preliminary evidence consistently reveals that vulnerable groups, chiefly sex workers and their clients are likely to be a major source of new infections.

Conclusions: The data from the observational studies will provide reliable information on the importance of individual risk factors for quantifying the risk of HIV transmission in the general population in Africa. It will also provide a platform for additional research, including intervention research and to use the epidemiological information for control programs.

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MEASURING NEW HIV-1 INFECTIONS IN SOUTH INDIA

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Plain Language Summary: India may soon have the greatest number of HIV-positive people in the world. Measuring how fast HIV spreads is important for designing prevention programs and allocating limited funds but can be difficult. It usually requires following groups of people for long periods of time and taking many blood samples. We aim to simplify the process by applying a new lab test which can estimate rate of HIV spread without the need for following people in time and would require only one blood sample. We will attempt to use this test to measure how fast HIV is spreading in Bombay, India.

Objectives: India may soon have the largest number of HIV-1 infected people in the world. Greater than 5.1 million people are currently infected. No reliable population-based estimates of HIV-1 incidence exist. The objective of our project is to develop the capacity to derive valid and reliable HIV-1 incidence estimates for volunteer counseling and testing centre (VCTC) attendees in Mumbai (formerly "Bombay"). A recently developed HIV-1 incidence assay, the BED capture enzyme immunoassay (BED-CEIA), identifies recently infected individuals using single serum specimens. It uses the principle of increasing antibody levels: people testing positive to a very sensitive assay but negative for a less sensitive assay are characterized as "recently" infected.

Methods: We will partner with the AIDS Research and Control Centre (ARCON) and the National AIDS Research Institute (NARI) in India to apply the BED-CEIA assay to measure HIV-1 incidence in high-risk populations in Mumbai, India. We have access to data from approximately 8000 persons self-reporting for testing in voluntary counseling and testing centres (VCTC). HIV-1 prevalence in this group approaches 20%. We will validate the BED-CEIA assay using specimens from 26 seroconverters infected during a 12-month period.(confirmed by a last negative ELISA test and a first positive HIV-1 ELISA result: two different ELISA kits confirm HIV status following WHO protocol for a confirming HIV-1 infection in a developing country) We will then apply the BED-CEIA incidence assay to all HIV-1 positive stored blood samples from 2000 to 2004. Detailed questionnaire data on attendees will help assess possible selection biases and permit quantification of correlates of HIV-1 incidence.

Results: Twenty-six seroconverters with last-negative and first-positive specimens within one year have been identified. Further specimens from 200 prevalent HIV-1 positive cases have been identified and demographic/risk information is available for the entire testing population. Further results will be announced.

Conclusions: If successful, incidence testing will be extended to all VCTC sites in Maharashtra generating the first population-based estimates of HIV-1 incidence and its correlates. Such estimates of HIV-1 incidence would provide benefits for surveillance, planning of vaccine trials, and evaluating preventative measures. Incorporating a validated incidence assay into HIV-1 surveillance in southern India will provide a cost-effective way of estimating incidence in disproportionately affected groups, such as commercial sex workers, and generate the first large-scale estimates of incidence in south India where the HIV-1 epidemic is growing fastest.

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STUDENT INTERNSHIPS IN CAMBODIA (I): AIDS AND ALCOHOL AT ANGKOR WAT, AND INCREASED RISKS FOR BEER PROMOTION WOMEN

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Plain Language Summary: Student interns and thesis researchers have helped build local research and intervention capacity in Cambodia, first with HIV/AIDS prevention programs and most recently studying alcohol risks in the workplace for beer promotion women, 20% sero-positive but left out of company ARVT plans. After drinking with clients, condom use is reduced and the risk of HIV transmission is increased. Drinking heavily in the workplace each evening may have long-term consequences associated with alcoholism. . Students interning in Cambodia gather data, help with health promotion workshops, can alert others on Canadian campuses that as consumers, they can pressure beer companies to provide safe, healthy workplaces worldwide (www.fairtradebeer.com), and can engage in fundraising.

Objectives: Using student internships and thesis projects to build local capacity for monitoring risk, creating and strengthening prevention and research programs dealing with the HIV/AIDS pandemic in Siem Reap, Cambodia.

Methods: Since 2002, students have helped, in a series of field visits, conduct baseline survey research, and qualitative interviews, develop research skills among local medical and NGO personnel, facilitate health education workshops, and evaluate a series of pyramidal peer-educator programs for groups at risk: married women, men, young tourist vendors, and the "beer promotion women" who sell international brands in restaurants (Heineken, Budweiser, Stella, Beck, Tiger); their HIV/AIDS prevalence rate is 20% (2000-4).

Results: International brewers treat their uniformed female sales force as "advertising/ promotion costs" rather than workers– they fall outside company health policy and receive no anti-retroviral therapy (ARVT). Two brewers began a "Safe Beer Selling "(SBS) program in 2004. Our interviews found no difference between women trained vs. untrained in SBS, with mean reported nightly beer-drinking with clients at 1 - 2.5 litres nightly, 28 days/month. We examine health consequences of immoderate drinking, plus increased risk for HIV/AIDS from non-use of condoms when inebriated. Neither company will adjust salaries to a fair wage, to help lower risk. One company began giving ARVT (Dec., 2003) to male brewery workers and management; none for women selling the beer.

Conclusions: Compared to Canadian counterparts serving the same brands in Ontario, Cambodian women are at major risk for HIV/AIDS and alcoholism. These breweries are now expanding in China with exponentially larger staffs of promotion women. Students interning in Cambodia help alert students on Canadian campuses that as consumers, they can pressure companies to provide safe, healthy workplaces worldwide (www.fairtradebeer.com), implementing their own international health and HIV/AIDS policies for the whole corporate family. "Beer promotion women" themselves are now active (www.beergirls.org).

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USING CHURCHES TO HELP SCALE-UP ACCESS TO HIV/AIDS PREVENTION AND CARE IN AFRICA

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Plain Language Summary: Presbyterian World Service & Development (PWS&D) works with partner churches in Africa and uses their grass-root networks to respond to the HIV/AIDS pandemic. We describe how the Presbyterian Church in Kenya is training church leaders to combat taboo, stigma and discrimination, and spread accurate information about HIV/AIDS. We describe how the Presbyterian Church in Malawi is mobilizing churches to incorporate HIV/AIDS themes into worship; organizing volunteers to support orphans, orphan families and provide home-based care for people living with AIDS; and providing loans to affected families for income-generating activities. These churches have large influence in their countries, with over 4 million members collectively.

Objectives: To help local church partners use their networks and human resources to respond to the HIV/AIDS pandemic by working in HIV/AIDS prevention, care and treatment in resource poor areas.

Methods: PWS&D provides grants to innovative HIV/AIDS programs from churches in Africa to help them scale-up their response to the HIV/AIDS pandemic. Educators and peers talk to communities about how HIV is, and isn't, spread, and call for an end to stigma and discrimination in the home, church and workplace. Volunteers provide quality home-based care for people who are ill, and help families care for orphans. Small loans and skills training help people infected and affected by HIV support themselves with small businesses. Agriculture and nutrition programs aim to help people stay healthy. We conduct systematic evaluations of the success of these programs through semi-structured interviews and on-site audits to determine participation, demographics and outcomes of cost.

Results: Orphans and orphan families are receiving care in community-based day care centers that are primarily run by volunteers. Trained home-based volunteers care for people in late-stage HIV/AIDS. Church ministers and leaders are providing accurate information regarding HIV/AIDS to help communities be supportive of people living with HIV/AIDS and combat stigma and discrimination in the church and community. The use of condoms for prevention is becoming more accepted in communities and among church members. Agriculture and income generating programs are assisting people living with HIV and AIDS to support their families and improve nutrition available to people living with HIV and AIDS.

Conclusions: With very little financial resources, churches in Kenya and Malawi are beginning to mobilize their human resources and grassroots networks to address HIV/AIDS with programs targeting education, prevention, care and treatment. Mobilizing churches and providing grants to cover costs can be an effective way to assist in the fight against HIV/AIDS in developing countries.

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"SMART" GATING STRATEGY FOR CD4 T-CELL ENUMERATION: AN AFFORDABLE UNIVERSAL APPLICATION

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Plain Language Summary: This study is proposing a low cost technique to perform CD4 enumeration. WHO has a global commitment to treat 3 millions HIV infected individuals by 2005. To meet this urgent global demand for reliable and affordable CD4 T-cell enumeration we propose to improve the panleucogate methodology by implementing the "Smart" gating strategy, a simple and universal instrument gating algorithm.

Objectives: Current guidelines in resource rich nations require a four-color single platform protocol for CD4 T-cell counting. In 2002 a protocol with more modest reagent requirements was introduced in South Africa by D. Glencross. This truncated method, PanLeucogate, represents a 60% reduction in reagent costs compared with the predicate method. In this report a further technical adjustment is introduced to the PanLeucogate protocol. The modification renders the PanLeucogate protocol more universally compatible with clinical flow cytometers. Objectives: to demonstrate how the modified PanLeucogate protocol can be rendered more suitable for across clinical platforms; to illustrate how this cost effective protocol can be integrated with a generic instrument setup to produce results that: (1) will not represent a compromise in quality of diagnostic services delivered during treatment of individuals living with HIV (2) will work with the two most frequently utilized clinical flow cytometers without complicating instrument operation.

Methods: The predicate method is a single platform protocol with Flow-Count absolute count calibrator beads and a set of four monoclonal antibodies CD45/CD3/CD4/CD8. The staining is performed by adding the conjugated antibodies to 100 µl of whole blood. The mixture is then lysed and fixed using ImmunoPrep reagents. The gating strategy utilizes a double anchor gate method with CD45/CD3. The PanLeucogate method is a double platform protocol where the hematology analyzer's WBC is multiplied with CD4 leucocyte percentage. Only two MAbs, CD45 and CD4, are required. The Glencross protocol requires a Forward Scatter (FS) instrument threshold. The modified protocol uses a CD45 fluorescence (FL) threshold. Ten specimens were analyzed on two high throughput flow cytometers, an Epics-XL and a FacsCalibur.

Results: Comparing both FS and FL threshold protocols to the predicate method on the Epics-XL with the Bland-Altman analysis we obtained standard deviation of 30.77 and 10.8 respectively. On the FacsCalibur where the comparison was restricted to the FL threshold protocol we obtained a SD of 12.10.

Conclusions: The data presented here, suggest that the "smart" gating strategy, the modified PanLeucogate protocol, renders a more universal and practical approach. It is imperative that larger multi-site evaluations are performed to confirm the above preliminary observation. This more universal application may bring about substantial savings in immunology laboratories when there is access to both types of conventional flow cytometers and a hematology instruments.

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BRING RELIABLE CD4 T-CELL ENUMERATION TO RESOURCE POOR REGIONS OF THE GLOBE: PART OF NHIL'S STRATEGY WITH INTERNATIONAL ALLIANCES

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Plain Language Summary: The National HIV Immunology Laboratory assists resource poor regions of the world with the implementation of CD4 enumeration, quality control and quality assessment by providing external quality assessment program and skill building workshops.

Objectives: Recently, some concerted efforts have begun to accelerate ART to reduce suffering from AIDS in Africa and Asia. WHO set an ambitious goal; to reach 3 million HIV infected individuals by 2005 in resource poor countries. In response, Canada pledged 100 million dollars to help WHO. Since 1996, the National HIV Immunology Laboratory (NHIL) has been making a significant contribution to improve the quality of CD4 T-cell enumeration in resource poor regions of the world. NHIL provides as a free service an international quality assessment program for CD4. With the help of WHO, NHIL offer skills building workshops to introduce quality assessment for T-cell subset enumeration. The first phase consists in transferring the technology to the selected trainers from a region. Second, the new trainers transfer the essential skills required to the local laboratory managers and technologists. The objective is to introduce effective infectious immunology laboratory infrastructure in resource poor region of the globe. To help with the rapid implementation of ART by supporting internal and external quality control program for CD4 T-cell immunophenotyping monitoring antiretroviral therapies against HIV.

Methods: Initial contacts with a regional office are usually made after a referral by WHO or CDC to help with ART monitoring for a resource poor region. Then, the regional requirements are identified and expectations resulting from the skills building workshop are identified. Next, the most suitable candidates are selected based on their background, capacity to teach and current level of involvement with the skills building workshop. The workshop is then adjusted both in terms of subject matter and length. One or more conference call is arranged to work out all the logistics of the workshop in Ottawa. This can include arrangement for visa, security clearance and required vaccination. The logistic of the regional workshop that will be conducted by the trainers for the first time is developed. The skills building workshop for the team leaders include a pre and post test and a certificate of performance. Within a month, the new instructors, helped by a coaching team, delivers the first local workshop.

Results: Several skills building workshops were organized for the regional instructors in Spain, South Africa, Trinidad and Tobago, Barbados, Senegal, Russia, Thailand and Ivory Coast. The regional skills building workshops conducted by the regional instructors took place in Ivory Coast, South Africa, Trinidad and Tobago.

Conclusions: The two phase technology transfer seems to be relatively cost effective.

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A WIDE CHOICE OF INSTRUMENTATION BUT ONLY A FEW VALID OPTIONS FOR CD4 T-CELL ENUMERATION

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Plain Language Summary: Establish the criteria essential to choose the most appropriate technology to perform CD4 enumeration.

Objectives: Current guidelines in resource rich nations require a five-reagent, four-color protocol for CD4 T-cell counting. There is a desperate need for more affordable tests on three continents. Recently, a number of new protocol/instrument combinations appeared with more modest reagent requirements. With some new innovative methods the reagent requirements are reduced by 80% compared to the predicate method. Instrumentation price spread is between $15,000 and $150,000 and for reagents from $1.50 to $100.00 per test. With such wide choice of products, it is important to select wisely. The objective is to disclose what tools and knowledge are essential to make evidence based decision when selecting T-cell subset enumeration technology for a resource poor region.

Methods: The essential criteria for CD4 T-cell immunophenotyping must be established. It must include a diverse collection of information as evidence for decision making. (1) Population demography: are all blood samples from adults or they will include pediatrics, (2) is it likely that the catchments area will expand for the region within two years (to centralize or decentralize), (3) minimum laboratory environmental requirements need consideration including secure space and staff bio-safety issues, (4) is the method compatible with available external quality control programmes, (5) is there data available about assay precision, accuracy and reproducibility (PAR).

Results: It is obvious that if your region does not have an airport you should not select an airplane as a mode of public transport, unless you can justify the additional cost of construction and maintenance of an airport. This analogy explains the importance of the rationalization exercise for identification and elimination of inappropriate options. The response to the above five questions will determine the type of the instrument and protocol your region requires. If your laboratory does not have the capacity to perform PAR, please demand results from a multi-site evaluation with independent data analysis where the population and disease demographics are the same or similar to yours.

Conclusions: Until recently, to deal with these formidable challenges, there were no comprehensive skills building workshops available for health care scientist from resource poor regions. With the help of WHO and other international agencies the National HIV Immunology Laboratory sets up technology transfer workshops. However they are infrequent and often inaccessible to those who would benefit from them the most.

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AN EVALUATION OF THE NUTRITIONAL STATUS OF PERSON INFECTED BY HIV-AIDS: THE CASE OF NGAOUNDERE PROVINCIAL HOSPITAL-CAMEROON

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Plain Language Summary: Morphologicals, haematologicals and biologicals alterations in the blood of persons infected by HIV-AIDS have been described by certain studies carried out in and out of Africa in AIDS patients.

Objectives: The objective of our study is to determine the nutritional status of an AIDS infected person in sub-Sahara in order to facilitate a proper care taking by evaluating the anthropometrical, bio-chemical (creatinin, cholesterol, transaminase), biological (red and white blood cells) perturbation parameters.

Methods: It implies a study of very talking case in which 100 persons (44 men and 56 women) did participate. These persons were given questionnaires in which information on the civil, chemical states, the anthropometrical parametres and the diet of the last two days were asked. Only those of the persons who gave their accord were retained. Blood specimen was taken for HIV-AIDS screening test (Elisa and Western blot) and to dose the transaminases, cholesterol, creatinin by spectrophotometer. Both the weight and skinfold were taken using the Holtain calliper.

Results: The results of this study revealed 72 persons infected and 28 negative cases. The transaminases increased enormously ($p<0,000003$) in the course of the infection. This increase must either be the consequence of a co-infection VIH-VHB or VIH-VHC responsible of the hepatological cytolysis or the action of opportuned infections. In PLHIV, the increase of transaminase which is two to tree times superior to the normal ($p<0,05$) translates not only a tissue necrosis but equally reveals a steatosis, a fibrosis and a hepatic cirrhosis. HIV infection and especially the presence of signs of an opportuned infection which provokes a muscular dystrophy revealed a significant drop of the creatinemia ($p<0,05$). The seropositive persons suffer from hypocholesterolemia which can have a serious effect on the maintenance of their health, ponderal deficit compared to the initial weight ($p<0,05$), and present a BMI and degree of skinfold which attests denutrition. The anthropometical parameters are influenced by the diet and the serological status. Some profound modifications in the composition of surrounding blood cells (leucopeania, aneamia) in the infected persons ($p<0,05$). The infected women present greater importance ($p<0,05$) of energy needs than the non-infected. Certain signs of opportuned illnesses lead to greater energy deficit than others; particularly the case of tuberculosis as a characteristic sign and cough as sign associated with HIV infection.

Conclusions: Nutritional support needs to be given to PLHIV and, in an holistic manner, in complement of all others treatments.

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PREVALENCE OF HIV AMONG WOMEN REQUESTING POST-ABORTION CARE IN EDO STATE, NIGERIA

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Plain Language Summary: Women requesting post-abortion care may be more likely to practice unprotected sex, and therefore may be at higher risk of HIV. To date, there is limited substantive data on the prevalence of HIV in women experiencing abortion in Nigeria.

Objectives: The objective of this study was to determine the prevalence of HIV among a cohort of women requesting post-abortion care in Benin City, Edo State, Nigeria.

Methods: A protocol was designed whereby all women undergoing post-abortion care at the Women's Health and Action Research Center (WHARC) were offered voluntary Counseling and Testing (VCT) for HIV. The Center adopted a policy of universal testing for these women, but with opportunity given to those not wanting to be tested to opt out. Testing was with a rapid screening test, followed by confirmation with ELISA. All HIV-positive women were offered treatment and follow-up care afterwards.

Results: Between June 1, 2001 and January 31, 2004, 274 women underwent post-abortion care at the Women's Health and Action Research Center. Of these, 255 (93.1%) accepted universal testing for HIV. Of the 255 acceptors, 19 (7.5%) proved HIV sero-positive. Among 19 sero-positives, 18 were unmarried; 6 were aged $\leq$ 20 years; two reported failure of emergency contraceptives; while none had ever used barrier contraceptives.

Conclusions: The results indicate an above national average prevalence of HIV among women seeking post-abortion care in Edo State. Acceptance of HIV testing under a regime of universal testing with the option to opt out is high among women seeking post-abortion care in Edo state. These results are useful for designing programs for the prevention of HIV, and suggest the need to intensify efforts at promoting dual contraceptive use among women in Edo State

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HIV TESTING AMONG PREGNANT WOMEN IN ONTARIO: RESULTS TO JUNE 2004

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Plain Language Summary: In 1994, it was found that antiviral medications could reduce transmission from an HIV-infected women to her newborn infant. Nevertheless, uptake of HIV testing in pregnant women in Ontario remained low and in 1999 a new program was implemented to address this issue. As a result, HIV testing increased from 40% to 89%, with most public health units reporting high HIV testing rates. Since 1999, the program has identified 160 undiagnosed HIV-infected women. Though testing rate is high, efforts must be undertaken to improve uptake even further.

Objectives: Despite the favourable results of the ACTG076 trial announced in 1994, few pregnant women in Ontario were tested for HIV in the years following. In 1999, the Ontario Ministry of Health implemented a program to offer HIV testing to all pregnant women; we examined subsequent HIV testing patterns.

Methods: In Ontario, prenatal screening for HIV and for most other infectious markers is carried out at the Public Health Laboratory. We determined the number of pregnancies for which at least one test was prescribed and the proportion with an HIV test carried out through the prenatal or HIV diagnostic program. We also examined HIV test results.

Results: From January 1999 to June 2004, 791,457 women were tested for at least one marker during pregnancy (mean 3,350 per week). In the first quarter of 1999, 40% of pregnancies were tested for HIV; this increased to 89% by the second quarter of 2004. Of the 37 public health units in Ontario, 15 had HIV test uptake of 90% or greater, 15 had 85-89%, four had 80-84% and only one was below 80%. Though 89% of women were tested for HIV in the latest quarter analyzed, 84% had been tested during the pregnancy and 5% at some time previously. 216 pregnancies tested HIV-positive (rate 0.40/1,000), of which 160 were newly diagnosed. The annual number of newly diagnosed HIV infections increased from 10 in 1999 to 49 in 2003. From January to June 2004, 20 new infections were identified.

Conclusions: Uptake of HIV testing in Ontario improved dramatically with the implementation of the new screening policy in January 1999. The increase was partly due to a reminder memo sent to physicians in late 2001. Though most pregnant women are now being tested for HIV during pregnancy, an estimated 16% are not. An indirect analysis of HIV testing patterns by physicians suggested that about half of these were due to physician-associated factors. Further research is required to better understand the reasons for pregnant women not being tested for HIV. Since the program began, the identification of 160 previously undiagnosed infected women prevented approximately 40 infections among infants.

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MEDICAL RECORDS AND WOMEN'S SELF-REPORT ARE NOT RELIABLE SOURCES FOR DETERMINING WHETHER ANTENATAL HIV TESTING WAS DONE

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Plain Language Summary: We set out to find out whether medical records and women's self-reports can tell us whether or not an HIV test was done during pregnancy. Women were recruited for participation following labor and delivery at 3 Toronto hospitals from Medical records tended to underestimate the number of women who had been tested. Self-report tended to overestimate the number of women who had been tested.

Objectives: In preparation for a study that will offer rapid HIV testing during labor, we set out to determine whether the medical record and the self-report of a post-partum patient provide reliable information about whether or not antenatal testing has been done.

Methods: Women were recruited for participation following labor and delivery on the postpartum wards of three Toronto teaching hospitals from November 2002 to February 2004. Following informed consent, the presence or absence of antenatal HIV testing was assessed by three approaches: (1) interviews with women 4 to 162 hours post-partum; (2) review of the labor and delivery charts; and (3) Provincial Laboratory HIV Testing Database and Prenatal Testing Database.

Results: 446 women were approached for participation, and 299 consented (67%). All women had at least one antenatal care visit, and 92% had antenatal records on their labor and delivery charts; 55% had documentation of HIV status on their charts; 46% had documentation that HIV testing was performed; and 46% had documentation of HIV test counseling. In interviews, 74% recalled their provider talking to them about HIV testing and 73% reported having an HIV test during this pregnancy. Laboratory test results in the two laboratory databases could be found for 66% of these women within one year of their delivery date. Medical records tended to underestimate the number of women who had been tested. Self-report tended to overestimate the number of women who had been tested.

Conclusions: In this group of women, health records and patient reports are both unreliable for determining who has had HIV testing and who has not had testing during pregnancy. This raises a challenge for offering rapid testing during labor and delivery.

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TESTING WOMEN AS MOTHERS: "IT WASN'T PRESENTED IN A WAY THAT WAS SAYING, WE WANT TO GET HIV TESTING SO THAT YOU CAN BE AWARE IF YOU'RE HIV-POSITIVE – IT WAS SO THAT THE BABY IS SAFE."

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Plain Language Summary: The established Canadian principles of HIV counselling and testing require that a person must give their informed consent to HIV testing and must receive counselling and education before and following testing. Working with a group of 57 pregnant women in Ontario, I learnt that that these principles are not always followed when HIV testing is carried out in the prenatal period. I conclude that it is very important that pregnant women receive the highest standards of HIV counselling and testing as, for pregnant women testing HIV positive, this is the first step in accessing interventions to take care of their own health and that of their unborn children.

Objectives: In the context of increasing rates of HIV infection among women world-wide, advances in prophylactic treatment and medical interventions with the potential to virtually eliminate HIV mother–to-child-transmission (MTCT) have served as a catalyst for policy and programme development aimed at increasing the number of women who test for HIV in their pregnancy. However, no other prenatal screening test reveals the presence in an asymptomatic pregnant woman of a chronic disease for which there is no cure. The objective of this paper is to examine whether a pregnant woman's rights to the established principles of HIV counselling and testing are maintained in the prenatal context.

Methods: Semi-structured interviews with a diverse group of 57 pregnant women whose responses were audio-taped, transcribed verbatim and interpreted using thematic analysis.

Results: Many pregnant women received little or no pre- and post-test counselling resulting in the inability to give or obtain informed consent to testing, inadequate preparation for the return of a positive result and loss of an often unique opportunity to learn of HIV prevention strategies. For many pregnant women the HIV test was largely transformed in the prenatal context from a specific diagnostic test with significant consequences for the woman testing positive, to a routinised component of good, prenatal care - a component the pregnant woman had little option but to accept.

Conclusions: Prenatal HIV testing (PHT) does not, in and of itself, prevent or reduce MTCT. Among prenatally diagnosed HIV+ women, reduction of MTCT takes place through the body of the pregnant woman. It is essential therefore that pregnant women receive the highest standards of HIV counselling and testing as the first steps in accessing interventions to take care of their own health and that of their unborn children. From the perspectives of the pregnant women in this study, PHT policy formulation and enactment works to construct a higher emphasis on the HIV prevention needs of mothers rather than of women.

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PERINATAL EXPOSURE TO ANTIRETROVIRAL THERAPY IN INFANTS BORN TO HIV SEROPOSITIVE MOTHERS: EVALUATION OF TOXICITY USING MITOCHONDRIAL DNA AND LACTATE LEVELS

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Plain Language Summary: While the use of antiretroviral therapy (ART) in pregnancy has significantly decreased rates of mother-to-child transmission of HIV, many questions remain regarding the safety of these therapies, and their potential impact on the uninfected infant. A number of adverse effects such as hyperlactatemia, believed to be related to mitochondrial toxicity, have been observed in infants exposed to ART pre and post-natally. Nucleoside analogues such as AZT inhibit the mitochondrial polymerase gamma, leading to mitochondrial DNA depletion and mitochondrial dysfunction.

Objectives: To investigate the impact of combination ART exposure in utero and in the perinatal period on infants born to HIV infected mothers, using a novel quantitative assay for mitochondrial DNA (mtDNA) and comparing this to venous lactate levels and clinical signs.

Methods: This is a prospective cohort study. Study subjects (N=50) will be recruited from The Hospital for Sick Children in Toronto and British Columbia Children's Hospital. Mitochondrial toxicity in ART-exposed infants is assessed by quantifying peripheral blood mtDNA and lactate levels longitudinally, using blood samples (≥350 uL) collected at 0-3 days, 4, 8, 12, 26 weeks of age. MtDNA from infants exposed to ART from conception will also be sequenced and compared to controls. Healthy controls will be recruited from newborns at BC Women's Hospital (N=10) and from infants seen in the general surgery clinic at The Hospital for Sick Children (N=40, 10 for each age group: 4, 8, 12 and 26 weeks).

Results: As of September 24th 2004, 20 study subjects have been enrolled from BCCH, and 17 from HSC (total =37/50), of whom 9 have reached 26 weeks of age. All 10 newborn controls and 13/40 older children have also had samples collected. The method to PCR and sequence the 16.5kb mtDNA genome has been developed. We anticipate enrolling the remainder of the study and control subjects over the next 6-12 months.

Conclusions: By monitoring longitudinal changes in mtDNA levels, in combination with blood lactate levels and clinical assessment from birth to six months, it may be possible to determine the timing and exposure associated with the development of nucleoside-induced toxicity. The results of this study will provide further knowledge regarding the potential adverse effects of ART on children born to HIV-infected mothers.

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PERSPECTIVES ON PARENTING: EXPERIENCES OF HIV+ PARENTS FROM SUB-SAHARA AFRICA AND THE CARIBBEAN

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Plain Language Summary: This presentation will highlight the experiences of parents living with HIV who are from Sub-Sahara Africa or the Caribbean and provide insight with respect to their priorities and concerns as parents living with HIV/AIDS in a new country. We ultimately want to use these finding to develop consumer-informed strategies to more effectively meet the unique needs of parents and children who move to Canada from these regions.

Objectives: Our objectives are: 1.1 Illuminate the role of beliefs and customs from participants home country/community in current daily routines and parenting practices in Canada; 1.2. Learn about how these parents handle disclosure of HIV status, particularly with their children; 1.3. Provide insights into how service providers might more effectively meet the needs of these parents and their children

Methods: In this qualitative study, drawing on both grounded theory and participatory research methods, we have interviewed over 20 parents living with HIV/AIDS who have settled in Ontario from Sub-Sarhara Africa or the Caribbean. These interviews are transcribed and analyzed using a constant comparison method frequently used in grounded-theory studies. Our research is guided by an advisory committee with service providers and consumers representing the communities in this study.

Results: Our results to date show that HIV+ parents experience a number of stressors upon moving to Canada, including for many, the discovery of their HIV status. Emergent themes include: Personal experiences such as stigma and fear about HIV in their communities and emotional distress and for some contemplation of suicide in the early stages following diagnosis; Socio-cultural strains in adapting to life in Canada and dealing with systems that are not set up to respond to families in culturally sensitive ways; and lastly parenting strains in trying to preserve values and approaches from their home countries while helping their child fit into life in Canada (such as issues of independence, respect for elders, disciplining practices), concerns dealing with HIV-related issues with their child (particularly a preference to protect their child from this knowledge), and drawing strength from their role as parents in dealing with the complexities of their life.

Conclusions: This presentation will provide researchers, service providers and PHA's with valuable insights about the experiences of parents from HIV endemic countries, their priorities and struggles as well as preferences with respect to service delivery

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CANADA PENSION PLAN AND PRIVATE INSURANCE DISABILITY POLICIES AND PRACTICES AS THEY AFFECT PEOPLE LIVING WITH HIV/AIDS (PHAS)

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Plain Language Summary: The Canadian Working Group on HIV and Rehabilitation commissioned an environmental scan to determine current policies and practices of the Canada Pension Plan Disability (CCP-D) Program and the private insurance industry relevant to people living with HIV. The experiences of PHAs in accessing and maintaining disability income supports vary widely. Many PHAs fear the potential loss of disability income supports; this fear may act as a barrier to work and active living. HIV is an episodic disability for many PHAs. Further study is required to understand how disability policies and procedures, and PHA perceptions of them, may inhibit return to active living.

Objectives: The project examines policies and practices of the Canada Pension Plan Disability (CCP-D) Program and the private insurance industry relevant to people living with HIV. The research examines the formal rules, regulations and practices of disability plans as well as the lived experience of those accessing them. This environmental scan is intended to help shape a second phase of research to further assess the strengths, weaknesses, barriers, gaps and disconnects of these policies and practices.

Methods: This project consists of the following components: I. A literature review. II. Qualitative key informant interviews with individuals including: people living with HIV; policy makers within government and private insurance industry; program staff within CPP-D, insurance industry and AIDS organizations; and others such as physicians, vocational rehabilitation specialists, social workers, etc.

Results: The scan finds that the experiences of people living with HIV in accessing and maintaining disability income supports vary widely. Variations depend on factors such as how recently people became disabled, the severity and episodic nature of their illness, the thoroughness and kind of information provided by themselves and their health care providers, literacy and assertiveness levels, and access to case workers or advocates. Experience with private insurance disability benefits also varies widely by the precise nature of the policy. Many PHAs experience fear about the potential loss of disability income supports and that this fear serves as a barrier to returning to work and active living. Moreover, the scan concludes that such fears – whether based in actual policy or based in misinformation – inhibit many people living with HIV from exploring whatever flexibility their disability insurers may indeed offer to those desiring rehabilitation.

Conclusions: The scan concludes that further study is required to understand how CPP-D and private insurance disability policies and procedures, and perceptions of them, may inhibit or facilitate return to active living. Further research should also identify strategies to overcome those real and perceptual barriers.

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PRACTICAL TOOLS FOR SURVIVING AND THRIVING WITH ONGOING LOSS: AN INTERVENTION FRAMEWORK FOR LONG-TERM MULTIPLE LOSS SURVIVORS

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1-AIDS Bereavement Project of Ontario; 2-Rainbow Tribe Consulting - Community Synthesis & Evaluation Insight; 3-St. Michael's Hospital, Inner-City Health Research Unit;

Plain Language Summary: In 2002-3, the AIDS Bereavement Project of Ontario conducted a community-based research project to develop an intervention framework, which involved a series of 2-day retreats with 67 long-term survivors (LTS) of HIV/AIDS across four pilot sites within the province of Ontario. Tools were developed to assess the impact of AIDS-related multiple loss and to address the experience of individual and communal losses. Our evaluations demonstrate that participation in retreats substantially improved quality of life and well-being. Implications for ASO frontline staff and volunteers and health and mental health care practitioners across the continuum of care are outlined in the intervention framework.

Objectives: To understand and document the experience of long-term multiple loss survivors of HIV in Ontario through the development of an intervention framework; with the aim of improving the quality of life and well-being of persons living with HIV/AIDS.

Methods: In 2002-3, the AIDS Bereavement Project of Ontario conducted a community-based research project to develop an intervention framework, which involved a series of 2-day retreats with 67 long-term survivors (LTS) of HIV/AIDS across four pilot sites. ASO Support Workers conducted recruitment, participated and coordinated follow-up peer support activities. Retreats consisted of a loss assessment tool, presentation and materials about AIDS-related multiple loss theory, facilitated group exercises. Impact of the retreat was evaluated using a multi-method pre/post self-completed questionnaire design and qualitative reflection tool: The Tree of Life. Data were analyzed using matched-paired analysis to determine the initial and sustained impact of retreat participation on various measures of depression, community involvement, social engagement and re-investment. Findings were disseminated through OAN Skills Building and PHA Programs.

Results: Participants report experiencing an average of 148 AIDS-related deaths in their life; with substantial physical and emotional effects related to their grief/loss including depression, anxiety, stress, anger/frustration, and hopelessness. At baseline, participants indicate: illness fatigue, being 'worn down', 'beaten' by HIV, and isolation/withdrawal as a means of coping. At three weeks and three months following the retreat indicate participants feel less depressed, less anxious and less guilty for being alive. Practical tools for understanding and working with complex individual and community losses are outlined in the intervention framework.

Conclusions: Many PLWHAs and affected individuals have experienced community devastation due to HIV/AIDS. Assessing and addressing the impact of AIDS-related multiple loss requires specific tools and unique models to support people at every stage, from loss, through re-creation of self to re-investment; and concerns support workers in ASOs, volunteers, and care and treatment practitioners across the continuum of care.

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ILLNESS-INDUCED INTERFERENCE WITH LIFESTYLE, ACTIVITIES, AND INTERESTS IN HIV-INFECTION

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Plain Language Summary: HIV-infection is a chronic life-threatening condition that can significantly disrupt lifestyles by interfering with valued activities and interests. This study found that fatigue, cognitive symptoms and HIV disease severity are associated with lifestyle disruptions in HIV/AIDS.

Objectives: HIV-infection is a chronic life-threatening condition that can significantly disrupt lifestyles by interfering with valued activities and interests. We examined the extent to which symptoms (fatigue, depression, cognitive, and medical) and neuropsychological impairment and markers of HIV disease affect 13 life domains: health, diet, work, active and passive recreation, financial situation, relationship with partner, sex life, family relations, other social situation, self-expression/self-improvement, religious expression, and community/civic involvement.

Methods: 360 adults (95% male) with HIV-infection (predominantly with mild symptomatic conditions or AIDS), completed a neuropsychological examination, the Beck Depression Inventory (Beck et al., 1993), the Patient's Assessment of Own Functioning Inventory (Chelune et al., 1986) and the Illness Intrusiveness Ratings Scale (Devins et al., 1983). Sample mean age and education were: 41.2 (SD=8.3) and 14.1 (SD=2.8), respectively. Multiple regression analyses examined the independent relations between markers of HIV disease, symptoms and neurobehavioural complications with total and factor-analytically derived illness intrusiveness dimensions.

Results: Fatigue, cognitive and medical symptoms, and HIV disease severity were significantly ($p<0.05$) and uniquely correlated with total intrusiveness ($R2=0.43$). Disruption in "instrumental" life domains (e.g., work, recreation, and health) were significantly correlated with fatigue, cognitive and medical symptoms, and HIV disease severity; as disruption in "psychosocial" functioning (e.g., civic involvement, self-expression, and social relations) was significantly correlated with depression, fatigue and HIV severity; and disruptions with intimacy were related to fatigue and increased cognitive symptoms only.

Conclusions: Fatigue, cognitive symptoms and HIV disease severity are associated with lifestyle disruptions in HIV-infection. Follow-up studies are underway to evaluate its utility to change with HAART and behavioural interventions.

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IMPACT OF ANTIRETROVIRAL THERAPY ON QUALITY OF LIFE

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Plain Language Summary: We conducted 5 focus groups with treatment-experienced HIV positive participants in order to identify the impact of antiretroviral therapy on quality of life. We identified key quality of life impacts from medication-taking that have not been well-described. Usual methods for measuring quality of life may miss important consequences of living HAART medications.

Objectives: To identify the impact of antiretroviral therapy on quality of life for those living with HIV infection

Methods: Using focus groups, key informants and sampling to informational redundancy, we elicited discussion regarding the impact of antiretroviral therapy on quality of life. An interview guide was developed and field-tested through in-depth interviews with five key informants (3 patients, 2 providers). A purposive sample of participants with a wide range of experiences with antiretroviral regimens was obtained using study inclusion criteria of: (1) having received HAART for at least 2 years; and (2) having received at least 1 prior HAART regimen for a minimum of 4 months. Data were audiotaped and transcribed verbatim. The transcripts were reviewed independently by two researchers coded iteratively using N.Vivo 2.0

Results: Five focus groups were conducted over July 2004-August 2004. A total of 37 participants (24 men and 13 women) took part in the focus groups. The mean age of participants was 44 yrs (range 25-68), the mean number of years since HIV diagnosis was 10 years (2-19). 30% of participants were receiving PI based regimens, and 49% were receiving NNRTI based regimen. Traditional quality of life domains were identified in addition to discussion around stigma, discrimination, shame, isolation, rejection, self-esteem, anger and punishment. Emerging themes were the concepts of (1) Trade-offs, being alive meant accepting the loss of important quality of life determinants, (2) Ripple-effect of medication toxicities (3) Ripple-effect of medication-taking and management, (4) Opposition between obtaining drug benefits and return-to-work, (5) Feeling life had become suspended or "in-limbo" without a clear view to future goals, expectations and planning, and (6) The importance of a good relationship with HIV providers.

Conclusions: There are downstream consequences of antiretroviral medication-taking on quality of life that go beyond the traditionally prioritized outcomes of viral load, CD4, slowed progression of disease, reduced mortality. These consequences have not been previously well-described. Usual methods for measuring quality of life may miss a number of important consequences of living HAART medications.

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THE HEALTH AND QUALITY OF LIFE OF HIV/AIDS CLIENTS WHO BECAME VOLUNTEERS: A 1 YEAR FOLLOW-UP SURVEY

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Plain Language Summary: Many volunteers begin as clients of the ASO. We were interested in understanding the changes in health and quality of life of clients/volunteers over a period of one year. The mental health of 65% of the clients/volunteers improved or stayed the same. These clients/volunteers reported a better quality of life that included better physical function, role function, social function, increased energy and less distress regarding their health. We concluded that the experience of volunteering contributed to a better quality of life for many clients, but not all.

Objectives: This study was designed to address the changes in health and quality of life experienced by PWA volunteers after the transition from client to volunteer.

Methods: A cross-sectional survey of clients who were volunteers was undertaken at baseline and one year after the initial survey. At follow-up, the clients/volunteers were clustered into 3 groups: those whose mental health deteriorated, did not change or improved. They were compared on their health, quality of life and future plans. In addition, the changes in health and quality of life were assessed for the clients/volunteers at the beginning of the study and one year later.

Results: The mental health of 65% of the clients/volunteers improved or stayed the same at the 1 year follow-up. Those who reported improved mental health, also reported better physical function, role function, social function, increased energy, less distress regarding their health and an overall better quality of life compared to those who reported poorer mental health.

Conclusions: Clients volunteer for a variety of reasons. Whatever the reasons, the experience of volunteering contributed to a better quality of life for many clients.

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Substance Use

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ARE DRUG-USING NETWORKS FORMED BY INJECTION DRUG USERS (IDUS) TRULY RANDOM?

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Plain Language Summary: Most disease transmission has traditionally been conceptualised as occurring between an infected person and a randomly selected number of contacts. It is assumed that on average, all the individuals in a network (people joined by specific interactions) have a similar number of contacts which when plotted on a graph results in a bell curve. Do we really believe that IDUs transmit HIV through their drug using relationships at random? Frequent drug use has been associated with injection equipment sharing. Therefore, we examine the distribution of the frequency of injection which provides further insight into the transmission of HIV/HCV among IDUs.

Objectives: Complex networks have traditionally been modeled as random graphs where, on average, all the individuals in a network have a similar number of contacts. If the number of contacts is truly random, it results in a bell curve when plotted on a graph. Research has shown the distributions of many real world networks such as the World Wide Web, metabolic systems and sexual partners follow a power law, which unlike the familiar Gaussian distribution has no typical scale and is called scale-free. In a scale-free network, weakly infectious viruses can spread and prevail; a consequence of the fact that a small number of individuals have a large number of contacts. Frequent drug use has been associated with sharing of injection equipment. Therefore we describe the observed frequency patterns of drug injection among IDUs in order to determine if the distribution of drug injection equipment sharing networks may contribute to the transmission of HIV and HCV among IDUs.

Methods: Data used for this investigation is from a cross-sectional pilot study undertaken among Winnipeg IDUs (n = 142) in 2003. The cumulative frequency of the number of times respondents injected over a 1-year period is plotted on a graph with logarithmic axes to determine the type of distribution. In such a graph, a power-law distribution will take the form of a straight line. Best fit was determined using the correlation coefficient and the standard error of the estimate. Exponents were determined using regression techniques.

Results: 80% (n = 114) of IDUs reported injecting 2160 times a year or less. A small number of individuals (n = 10) reported injecting 4320 times a year or more (maximum 15000 times a year). The power law model provides a good fit (r = 0.99) for the available data however it should be noted that the small sample size may not provide a valid representation of the IDU population.

Conclusions: These results have important implications in that epidemics arise and propagate faster in scale-free networks, such as that suggested by our data. Randomly targeted interventions have little impact on the spread of disease in a scale-free network and fundamentally different interventions are required to reduce the transmission of HIV/HCV through sharing drug injection equipment. These preliminary analyses suggest that further investigation regarding the distribution of IDU networks is warranted.

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RISK CONDITIONS IMPACT INDIVIDUAL HIV PREVENTION PRACTICES OF OTTAWA INJECTION DRUG USERS

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Plain Language Summary: We interviewed 506 injection drug users (IDUs) in Ottawa and learnt that injecting with a used needle was significantly associated with testing positive for HIV infection. IDUs told us that they injected with someone else's used needle because clean needles are hard to get. We estimated that Ottawa IDUs needed 6.7 million needles in 2003 so that a clean needle could be used for each injection. However, only a small proportion of this total was actually distributed. We conclude that IDUs need increased coverage of clean needles so that they can keep themselves and others free from HIV infection.

Objectives: Research identifying sexual behaviours and injection practices associated with HIV among injection drug users (IDUs) has been vital in driving evidence-based interventions to promote individual behaviour change. However, individual behaviours may be mediated by the experience of externally constructed HIV-related risk conditions. The objectives of this study were to determine factors related to prevalent HIV infection and to situate these data in the context of structural conditions contributing to IDUs' risk of infection.

Methods: From October 2002 to January 2003, 506 street-recruited IDUs completed interviews; 485 consented to anonymous HIV saliva testing. Risk factors for prevalent HIV infection were determined using multivariate logistic regression. Annual number of sterile needles required by Ottawa's estimated IDU population (approximately 3,000) by drug of choice and injection frequency was estimated and compared with actual number of needles distributed in 2003 by Ottawa's needle exchange program (NEP).

Results: 54/485 IDUs (11.1%, 95% CI: 8.5–14.2) tested HIV-positive. Prevalent HIV infection was significantly associated with a history of sharing needles (AOR=2.6, 95% CI: 1.2,5.7). Further examination of this behaviour demonstrated limited availability of sterile needles: IDUs who had recently injected with used needles reported that sterile needles were "hard to get" (24%); NEP closed or mobile NEP not accessible (13%); and NEP hard to reach (6%). Findings were confirmed by modelled estimates of needle coverage: approximately 6.7 million injections took place in 2003, whereas 159,000 needles were distributed by the NEP, representing 2.4% of the estimated need for sterile needles in Ottawa.

Conclusions: The risk condition of inadequate needle coverage seriously compromises IDUs' capacity to inject safely. Until such risk conditions are modified through action in political and public health domains, HIV prevention programs focusing on individual behaviour change will not be sufficient to impact IDUs' risk of acquiring HIV.

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UNMET MENTAL HEALTH NEEDS AMONG IDUS IN METHADONE MAINTENANCE IN ONTARIO

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Plain Language Summary: Unmet mental health needs (e.g., anxiety, depression) present a challenge for low threshold methadone maintenance treatment (MMT) programs that are designed to reduce drug-related harm and HIV transmission. Using data from a qualitative study of four methadone programs in southern Ontario, we explore systemic, social and individual level barriers that prevent MMT clients from receiving mental health services. Access to these services is an important aspect of drug treatment and harm reduction. Mental health supports can help clients to stabilize their lives and enable development of new coping strategies to replace drug use.

Objectives: To understand the systemic, social and individual factors that contribute to unmet mental health needs among MMT clients and the implications for treatment outcome, including HIV prevention.

Methods: Data from semi-structured interviews with clients (n=64), workers (n=18), and physicians (n=14) at 4 low threshold MMT programs in Ontario, as well as site observations and documents, were collected and analyzed using qualitative methods.

Results: Program clients, staff and doctors identified barriers at the systemic, social and individual levels that prevented MMT clients from receiving mental health care: Systemic: The programs were not well supported by the mental health system. Only one program had a formal link to the mental health system that was facilitated by a mental health outreach worker. Few mental health resources were available onsite. While MMT physicians provided varying levels of primary care, they defined their role primarily as methadone prescribers and offered little or no mental health care or counselling. Referrals to community mental health services were also problematic. Community psychiatrists were reported to refuse MMT clients because they were deemed too complex to treat and/or patients were required but unable to abstain from all drug use as a precondition to psychiatric care. Social: MMT clients were unwelcome at other community programs (e.g., residential mental health program). MMT clients were stigmatized as "drug addicts" and considered 'risky', 'non compliant' and/or 'difficult'. Individual: The chaotic, complicated and often socially isolated lives of many MMT clients often conflicted with other appointment-oriented service providers and limited support from friends and family. MMT clients often missed appointments and/or became impatient with other service providers.

Conclusions: Clients, staff and doctors stressed the importance of including mental health care in MMT programs as these services would help clients to stabilize their lives and develop new coping strategies to replace drug use.

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CHANGES IN PATTERNS OF SUBSTANCE USE AMONG PARTICIPANTS IN THE POLARIS HIV SEROCONVERSION STUDY

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Plain Language Summary: The purpose of this analysis is to describe changes and differences in patterns of alcohol and drug use over time among participants enrolled in the Polaris HIV Seroconversion Study. Patterns of use and non-use of various substances remained the same over time for both HIV-positive and HIV-negative individuals. However, there was a decrease in heavy alcohol use and an increase in marijuana use over time. Fewer participants used cocaine, amphetamines and psychedelics between baseline and first follow-up, but there was no further change over time. There was a trend toward less heavy drinking among HIV-positive participants compared to HIV-negative individuals.

Objectives: To identify changes and differences in patterns of substance use over time among participants in the Polaris cohort.

Methods: Polaris is an ongoing longitudinal open cohort study of recent seroconverters and HIV-negative controls in Ontario. Participants are recruited through Ontario's HIV diagnostic laboratory, physicians, community organizations and media. Recent seroconverters (cases) were determined using HIV test results. Cases are gender-, risk-, and region- matched to HIV-negative controls using a 1:2 case-control design. A sample of 379 (144 HIV-positive and 235 HIV-negative) participants enrolled and followed between June 1998 and January 2004 was analyzed. Data sources included quantitative induction and follow-up interviews. Cases were asked about behaviour in the time prior to their first HIV-positive and last HIV-negative test, and controls were asked about an equivalent time period. At follow-up, participants were asked about behaviour since their last interview. Measures of substance use included use versus non-use of substances -- alcohol, marijuana, cocaine, crack-cocaine, amphetamines, psychedelics, and ecstasy. GEE logistic regression was used to describe changes in patterns of drug use over time after controlling for participants' characteristics (i.e. HIV status, MSM versus non-MSM, gender and age at induction). Results reported are significant at p-value <0.05.

Results: Patterns of increase and decrease in substance use primarily remained consistent over time for both cases and controls. There was a significant reduction in heavy alcohol consumption, and a non-significant increase in marijuana use over time. A significant reduction was found between induction and first follow-up for cocaine, amphetamine and psychedelic use, but there was no further significant change over time. With respect to differences between groups based on participants' characteristics, there was a trend towards a greater reduction in heavy drinking after induction, and a faster rate of decline in use, among HIV-positive individuals compared to HIV-negative controls. However, HIV status was not a strong predictor of other substance use. MSMs were more likely to be moderate users of alcohol and to use ecstasy than non-MSMs, but were less likely to use cocaine and crack-cocaine than non-MSMs. Males were also more likely to report cocaine and crack-cocaine use compared to females. Younger participants were more likely to drink alcohol heavily, and use cocaine, amphetamines, psychedelics and ecstasy relative to older participants.

Conclusions: HIV seroconversion does not appear to precipitate any long-term change in use and non-use of substances. Further analysis will examine frequency of use and simultaneous drug use to ensure that substitution (complementary) effects are neither overlooked nor mistaken for change.

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Vulnerable Populations

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A RESEARCH AGENDA TO EXPLORE THE ROLES OF STIGMA, DISCRIMINATION AND CULTURAL CONSTRUCTS THAT CONTRIBUTE TO THE BURDEN OF HIV AMONG AFRICAN AND CARIBBEAN PEOPLE IN ONTARIO

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Plain Language Summary: The information available in Ontario and Canada heralds the urgency to determine what factors put people of African and Caribbean descent in Ontario and Canada at a higher risk for HIV and to understand barriers these individuals may face in reducing their risks. There is little empirical research evidence that has been gathered so far. There is a need for evidence to improve our understanding of HIV in this community and to guide HIV program planning in education and other health promotion interventions to reduce the burden of HIV in this community.

Objectives: To assess the role of self-stigma, stigma within the community and perceived discrimination from outside the community in contributing to individuals risk behaviours (including homosexual and bisexual risk behaviour), and access to HIV treatment, including testing, drug therapy initiation and adherence.

Methods: The proposed study design will use the Grounded Theory approach and comprise of individual interviews. Individual interviews are preferred over focus groups as the study is exploratory and themes have not as yet been developed that could form the pivotal point of a focus group discussion. Further, some of the information sought will be of an intimate nature and will not be appropriate for group discussion. Data collection will be stratified to collect an equal proportion of people from African and Caribbean communities (20 each). Each community strata will comprise equal numbers of males and females. The focus will be on individuals 18 – 49 years, HIV positive, who immigrated within the last 10 years and are able to communicate in English

Results: It will be used to examine the individual's perceptions of societal beliefs and attitudes on HIV before and after HIV seroconversion. The interview will also look at sexual practices within the context of being HIV positive and the impact of HIV on the individual's life and that of their social network. Information on bisexuality, gay sexuality and other emerging themes will be explored

Conclusions: The objectives are timely to address HIV prevention, care and treatment for the burgeoning problem of HIV in inner city residents, many of whom are African and Caribbean people. Although the prevalence of HIV is still higher among gay men in Ontario, recent trends have shown an increase in the percentage of new HIV infections occurring among the ethno-cultural population. The burden of HIV among individuals from endemic countries is not only economical but also social and acutely affects the whole community due to the way in which families are structured, patterns of immigration and existing discrimination that accentuates the disease. Also, the data show that women of African and Caribbean descent present a growing proportion of the new HIV infections in Ontario. Many of the paediatric infections still occurring in Ontario are born to women of colour. Thus the need to address this in counselling, testing, family planning and in the general health care setting is paramount. The empirical evidence that is needed to guide practice, treatment and care for those with HIV and in the special context of grappling with issues of employment, lack of cohesive social structure and heterogeneous discrimination is lacking. Thus this study will aid in finding ways of addressing HIV among African and Caribbean communities in Toronto.

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CONNECTING SEXUAL HEALTH AND CULTURE-SEXUAL HEALTH EDUCATION FOR IMMIGRANTS: A MODEL

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Plain Language Summary: A culturally sensitive, primarily visual tool to facilitate education of immigrant women and men in the sexual health area has been developed in response to the rise in sexually transmitted infections among immigrant populations. Includes tips and examples for ease of cross-cultural communication. This unique resource addresses the two most important health determinants resulting from the research conducted within the Regina immigrant communities, namely, culture and language. Thus the format and design reflect simplicity of language with the text supplemented by overheads (included). The model is divided into four sections consisting of values, culture, choices and access to local health services. Each section includes a guide for the facilitator in terms of questions to ask and points to remember. Cultural sensitivity and use of basic language is stressed. A new and unique resource for anyone in the education field.

Objectives: Immigrant Women of Saskatchewan, Regina Chapter received a grant from Health Canada to research, design and develop a model of sexual health education for immigrants. The health determinants addressed in the research were; social environments, personal health practices, coping skills, health services, gender and culture.

Methods: The methodology followed was that of a Community Consultation Model. 10 consultations were conducted with approximately 100 participants, 80 women and 20 men. Data was gathered through written surveys and oral discussion. Regina's ethno-cultural population is varied comprising of small pockets of immigrants from around the world. Careful consideration was given to including all racial and cultural groups as well as using inclusive and simple language during discussions.

Results: The results indicated that language and cultural sensitivity are the two most important health determinants for accessing sexual health services among immigrants. Of the 56 women respondents of written surveys, 40% indicated they were very or somewhat comfortable in English. 59% were interested in their sexual health but only 36% went to the doctor for regular check-ups. The surprise finding was that 33% said they would consult a medical professional for sexual health information rather than husband (28%). In oral discussions, birth control was the major area of concern for women while fears and cures of STI's predominated for men.

Conclusions: Immigrant women and men have different perceptions and needs. This approach to sexual health education is not only culturally sensitive but ensures the comfort level of the participants by careful dialogue of the "known" (culture and values) and the "unknown" (choices) to provide pertinent sexual health education.

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CANADIAN ABORIGINAL PEOPLE LIVING WITH HIV/AIDS (APHAS): CARE, TREATMENT AND SUPPORT ISSUES

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Plain Language Summary: HIV/AIDS has been and in all likelihood will continue to be a serious health concern affecting the Aboriginal (First Nations, Métis and Inuit) community (CIDPC, May 2004). It was against this backdrop that the goals of this study were first defined. With funding provided by the Canadian Institutes of Health Research and ethical review by Carleton University, this study seeks to document the extent to which services are meeting the needs of Aboriginal People Living with HIV/AIDS (APHAs), by geographic region, disease stage, gender, age and transmission category. As secondary objectives, deficiencies in the provision of services are identified and policy and/or practice recommendations developed.

Objectives: This study seeks to document the extent to which services are meeting the needs of Aboriginal People Living with HIV/AIDS (APHAs), by geographic region, disease stage, gender, age and transmission category. As secondary objectives, deficiencies in the provision of services are identified and policy and/or practice recommendations developed.

Methods: Using a participatory research design, and with assistance from 31 recruitment agencies, we provided APHAs from across Canada a self-administered mail survey, resulting in 195 responses (a response rate of 65%). Regional representation was achieved, and the survey sample includes Inuit, Innu, Métis, and First Nation APHAs representing the full cross-section of Canada's Aboriginal population in terms of gender, sexual orientation, age, and urban/rural residence. The questionnaire was designed to obtain relevant demographic information coupled with a list of possible services. Participants were asked to rate the extent to which needs were met by the services they used or needed. All responses were coded and entered into SPSS with the main types of analysis focusing on frequency and cross-tabulation. Tests of statistical significance were also preformed.

Results: Across Canada (all regions) the majority of APHAs (61%) use or need traditional services such as counseling with Elders, healing/sharing circles, traditional medicines and ceremonies, which reinforce a positive sense of identity and well-being. Indeed, recommendations for more Aboriginal front-line workers and expressions of need for emotional, mental and spiritual support were recurring themes within open-ended responses (25% and 12% respectively). Services provided by community and AIDS Service organizations (eg., buddies, drop-ins, etc.) are widely relied upon, particularly by urban APHAs who are HIV positive and beginning to experience health problems (82%). Notably, 43% of APHAs indicated that they will need to move or have already moved in order to be nearer HIV/AIDS services, and almost 10% of cases recommended that more support services be provided on Reserves and in rural or isolated Aboriginal settlements. That both traditional and community/ASO services received the highest numbers of unsolicited positive evaluations (72% and 64% of responses, respectively) is significant with respect to the future of care, treatment and support for APHAs in Canada. Conversely, although APHAs indicate that their needs are generally being met by both primary and secondary medical services, considerable logistical barriers (42-62%; eg., unavailability, transportation) and financial barriers (29-35%; limited coverage for fee-based services, eg. chiropractor, optician) are reasons commonly given for why needs are not being met. APHAs also indicate that HIV/AIDS stigma and racial discrimination among mainstream medical and social service providers continue to act as a barrier (20-30% of responses).

Conclusions: Analysis revealed the following; APHAs generally feel their needs are being met but there remains significant areas for improvement. For example, many felt that more and sustainable support services (e.g., drop-ins or support groups, etc.) were needed coupled with the expressed need to more aggressively attend to issues of cultural content in programming (e.g., access to elders, sharing circles and other traditional ceremonies, etc.). Often calls for support services and for cultural competence in programming go unmet by those with responsibility for obtaining funding for services. This has also not gone unnoticed by APHAs accessing HIV-related services who articulate similar calls for increased funding to support urgently needed HIV-related social and cultural supports. Acknowledgments: The research team and advisory committee members (Kim Thomas, LaVerne Monette, Gabe Saulnier, Henry Kudluk) acknowledge the collective wisdom of study participants.

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THE ACCEPTABILITY AND EFFECTIVENESS OF A INTERNET PEER SUPPORT OUTREACH PROGRAMME CALLED i-RICE FOR EAST AND SOUTHEAST ASIAN MEN WHO HAVE SEX WITH MEN (AMSM)

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Plain Language Summary: Stage 1: To study the characteristics of all i-rice users and their satisfaction with the i-Rice website. Stage 2: To study the lifestyle characteristics, risk behaviors, stressors, attitudes and belonging of Asian Men using i-Rice.

Objectives: The objective is to provide better service to AMSM. i-Rice was developed as an internet peer support outreach project aiming at empowerment education, STIs and HIV/AIDS prevention. This site promotes positive East and Southeast Asian images and healthy sexuality in providing knowledge and counseling. The second survey will provide us with information about the characteristics of AMSM using i-Rice.

Methods: Stage 1: All people using the i-Rice website are asked to participate in a 15 minute survey online asking questions about their satisfaction with the website, what kind of things would they like to see on this website, changes, what information was helpful/not helpful. Also included are questions about the i-rice user characteristics (ethnicity, age, education, immigration status, employment, relationship and health status). Stage 2: Willing participants who are Asian are asked to participate in either a personal or telephone interview about their risk behaviors, stigma, stressors, interacting and belonging.

Results: Preliminary results available 2005. The presentation will discuss the implementation of this survey on-line and the challenges encountered.

Conclusions: Preliminary findings will be available in 2005. The expected important findings are that ACAS will have a better understanding of the i-ricer user and tailor their prevention and outreach in the community to reduce risk behaviors.

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AN HIV RISK REDUCTION INTERVENTION WITH INCARCERATED YOUTH IN ONTARIO

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Plain Language Summary: This study determined the effect of an intensive educational and skills building program designed to reduce HIV risk among young offenders. Voluntary participants from secure custody facilities in Ontario were randomly assigned to one of three groups: an education group, an education plus booster group, and a no special program control group (with a shortened educational program offered at completion). Self-reports of attitudes, knowledge and behaviours with respect to HIV/AIDS were assessed. Differences between males and females were identified with respect to risk behaviours and how they responded to the intervention. There were several factors identified that could be helpful in future HIV educational interventions.

Objectives: (1) To evaluate the impact of a structured, comprehensive HIV risk reduction intervention on knowledge, attitudes and behaviours about HIV/AIDS in incarcerated youth by gender. (2) To determine predictors of success in increasing HIV knowledge, reducing high risk attitudes and decreasing high risk behaviours.

Methods: Adolescent males and females between the ages of 12-18 residing in closed custody facilities in Ontario were recruited. Voluntary participants were randomly assigned to one of three conditions: (1) educational intervention (2) educational intervention with two booster sessions and (3) no systematic intervention control (with condensed education session offered after last post-test). Baseline measures included questions on knowledge, attitudes and behaviours (KAB) toward HIV/AIDS, the Youth Self Report (YSR), and the Drug Use Inventory (DUI). Participants completed structured interviews at 1, 3, 6, and 12 months following enrollment in the study. Multivariate analysis of variance, and generalized estimating equation models were used to analyze changes in measurement over time. Linear regression analyses were used to examine predictors of success.

Results: 391 subjects were recruited; 102 females and 289 males. Risk behaviours were dramatically different from community norms. HIV knowledge and attitude scores increased for study participants. Differences were observed between males and females with regard to their prevention attitudes, condom attitudes and risk behaviour knowledge. Age, gender, self-esteem, severity of drug use and internalizing and externalizing psychological profiles were predictive of outcome.

Conclusions: This study targeted a high risk and hard-to-serve population. Participant feedback was enthusiastic. The intervention was successful in improving knowledge and attitudes immediately post intervention and several of these changes were sustained. This study has identified several predictors of success that could be helpful in future HIV educational interventions with youth.

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Policy Issues

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ONTARIO HIV TREATMENT NETWORK SCIENTIST CAREER FUNDING-DOES IT MAKE A DIFFERENCE?

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1-Ontario HIV Treatment Network, Toronto, Ontario; 2-HIV Studies Unit, University of Toronto;

Plain Language Summary: The OHTN Board of Directors was interested in knowing the value of the funding invested in career support of Ontario scientists. Staff carried out a survey and determined that scientists are able to spend 70% of their time on research and this benefits HIV research in the province by ensuring that research funds are successfully obtained, research is carried out and the findings are published and shared with policy makers and others who need the information to make decisions.

Objectives: A survey of current and former awardees was carried out to determine the value of OHTN career support awards in terms of scientific productivity, impact on the professional careers of individual awardees and impact on the HIV research environment in Ontario.

Methods: A questionnaire was designed to determine impact of the support on: current employment and academic status, scientific productivity, community interactions and impact on personal and professional decisions.

Results: 11/12 Career Scientists and 6/6 Scholars completed the questionnaire. All respondents hold university appointments and spend more than 70% of their time on HIV research with the balance devoted to clinical, teaching, laboratory or administrative duties. Awardees have successfully competed for peer-reviewed non-OHTN funding (>$1.6 million per Scientist, $400,000 per Scholar). Academic output includes more than 150 peer reviewed publications, 67 manuscripts in progress, and >330 scientific abstracts presented during the period of support. Other stated benefits included development of new collaborations, policy development opportunities, travel and international networking, promotion, and scientific development opportunities. Most respondents (13/17) indicated that holding an OHTN award had increased their ability and opportunities to work with community-based organizations (CBOs). Nearly all respondents (16/17) indicated that OHTN career support played a crucial role in enabling them to pursue their career in HIV research in Ontario. Protected research time enabled awardees to be productive, collaborative and creative.

Conclusions: OHTN investment in career support awards has had a major positive impact on the HIV research environment in Ontario. Protecting research time has significantly enhanced awardees' ability to compete for peer-reviewed funding, increased publication productivity and improve interactions with community-based organizations. Return for dollar invested is 2:1 for Career Scientists and 1:1 for Scholars. Further research is necessary to determine what effect the investment has had on HIV care and treatment in Ontario.

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ENHANCING DIAGNOSTIC DATA FOR HIV SURVEILLANCE: RESULTS OF THE ONTARIO LABORATORY ENHANCEMENT STUDY (LES) TO DECEMBER 2003

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Plain Language Summary: A new technique used on HIV positive specimens identifies recently HIV infected persons. Enhanced data on risk factors and adjustments for HIV testing time enabled population-specific estimates of new infections. HIV incidence (per 100 person-years) from 1999 to 2003 was: MSM 1.5, MSM-IDU 3.0, IDU 0.23. New HIV infections were similarly higher among Toronto and Ottawa MSM than elsewhere with trends over time stable in Toronto but increasing substantially in Ottawa. HIV infection was highest among Ottawa IDUs with reassuring decreasing rates in all regions over time.

Objectives: 1) To estimate and monitor trends in HIV incidence among persons undergoing HIV testing according to exposure category and region; 2) To improve risk factor information and HIV testing history using the LES study.

Methods: For all HIV-positive and a 1:200 sample of negative test results, we sent a questionnaire on HIV-related risks and previous HIV testing. We tested positive specimens by the detuned assay, a less sensitive version of the Abbott 3A11 HIV-1 and Vironostika EIA assays modified to detect recent HIV infection. Using enhanced data, we calculated HIV incidence for each exposure category and adjusted for HIV testing bias using newly developed analytic software.

Results: From October 1999 to December 2003, 4,465 patients were newly diagnosed with HIV; 78% of questionnaires were returned within 8 months. Risk data was provided on only 54% of laboratory requisitions. The proportions of tests among HIV-endemic and MSM categories were different based on data from the returned LES questionnaires compared to the laboratory requisition: HIV-endemic 27% versus 8% and MSM 38% versus 54%. Overall, HIV incidence (per 100 person-years) was: MSM 1.5, MSM-IDU 3.0, IDU 0.23 and persons with an HIV-infected or at-risk sexual partner of the opposite sex 0.08. Incidence was higher among Toronto and Ottawa MSM than elsewhere (1.9 in Toronto, 1.7 in Ottawa and 0.67 elsewhere) and among Ottawa IDUs than elsewhere (0.69 in Ottawa, 0.20 in Toronto and 0.17 elsewhere). Over time, HIV incidence among MSM has been stable in Toronto since 2000 but increased substantially in Ottawa from 0.0 and 0.25 in 1999 and 2000, respectively, to 1.8 and 1.4 in 2002 and 2003, respectively. Among IDUs, HIV incidence decreased in all areas, in Toronto from 0.25 in 2000 to 0.09 in 2003; in Ottawa, from high levels of 0.61 in 2001 to 0.29 in both 2002 and 2003; and rates elsewhere also decreased from 0.16 in 2000 to 0.13 in 2003.

Conclusions: The LES provided important data on risk and HIV test history and yielded estimates of exposure category-specific HIV incidence over time. The likely overestimation of the observed incidence was adjusted using a new analytic approach. No increasing HIV incidence among MSM in Toronto is reassuring despite the syphilis outbreak but sustained increase among MSM in Ottawa is of concern. Decreasing rates among IDUs in Ottawa and elsewhere is reassuring but need to be closely monitored.

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THROUGH A GLASS DARKLY: EVALUATION OF HIV/AIDS WEBSITE REACH, USAGE AND IMPACT

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Plain Language Summary: Using the Canadian AIDS Treatment Information Exchange (CATIE) Web site as a case study, this presentation compares and assesses of a number of different methods for evaluating an HIV/AIDS Web site. We discuss the limitations of different methods, and how and why we used more than one approach to draw conclusions about how a Web site was used, and with what impact.

Objectives: The Canadian AIDS Treatment Information Exchange (CATIE)'s bilingual Website (CATIE.ca) is a leading source of HIV/AIDS treatment information, having won international recognition from Médisite, Britannica's Internet Guide, the Lancet, and JAMA. With over 10,000 pages of content, CATIE.ca is Canada's largest online archive of HIV/AIDS treatment information.

Methods: In order to assess Website usage, reach and impact, CATIE conducted evaluation research using three methods: 1) aggregate server log analysis (1996-2003); 2) Website user survey (2003, n=263); and 3) key informant interviews (2002, n=41).

Results: Server log analysis provided indicators of site usage (24,000 user sessions/month), content access (most popular content: fact sheets and news), and historical usage trends. Challenges included defining 'page', excluding non-person visits, and interpreting ISP address labels. The Web survey provided indicators of who uses the site (36% people living with HIV/AIDS, 39% community caregivers, 14% health care providers), their geographical origin, how information is used (44% managing their health, 29% sharing with family/friends, 28% professional development, 23% research projects, 16% giving to clients), and site helpfulness (>86% found the site helpful or very helpful). Extracting meaningful information involved sampling challenges. Comparing survey results to log file analysis on overlapping indicators allowed independent verification. Both the user survey and key informant interviews found that users want: quality information, interactivity, regular updates, easy navigation and targeted information.

Conclusions: Due to inherent limitations in each methodology, multiple methods are needed to fully grasp reach, usage and impact of a Website. Mixed methods also give opportunities for triangulating methods in order to increase confidence. CATIE's experience has shown the ability of mixed evaluation methods to inform future Website development, and also offers useful benchmarks for other Websites.

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HIV & REHABILITATION: CREATING A SAMPLING FRAME OF SELECTED HEALTH CARE PROVIDER GROUPS FOR A NATIONAL SURVEY

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Plain Language Summary: A random sample of rehabilitation service providers who may or may not be working in the area of HIV/AIDS was drawn from mailing lists obtained from national professional organizations and supplemented with available provincial regulatory body mailing lists. Mailing lists for the known population of HIV specialists working in HIV/AIDS were obtained from HIV and/or national professional organizations, web sites, Colleges of Physicians and Surgeons, and snowball sampling. Cross-checking national lists with those of provincial regulatory bodies increased the number of rehabilitation professionals in the sampling frame. Snowball sampling is useful in obtaining mailing list information for some HIV service providers.

Objectives: To describe the creation of a sampling frame for a national cross-sectional survey of selected health care provider groups concerning rehabilitation services for people living with HIV/AIDS.

Methods: A Canadian survey was conducted between April – August 2004 with rehabilitation service providers and HIV specialists using the Dillman Tailored Design Method. A random sample of rehabilitation service providers (physical therapists, occupational therapists, physiatrists, and speech-language pathologists) who may or may not be working in the area of HIV/AIDS was drawn from mailing lists obtained from national professional organizations and supplemented with available provincial regulatory body mailing lists. Discrepancies between lists were corrected, and duplicate addresses removed. The known population of HIV specialists (physicians {general practitioners, infectious disease specialists and psychiatrists}, nurses, dieticians, pharmacists, social workers, and psychologists) working in HIV/AIDS was identified using snowball sampling.

Results: Total time required to assemble the final sample was five months. For rehabilitation service providers, nine of thirteen provincial-territorial regulatory bodies provided mailing lists to supplement national organization lists for physical therapy (PT) and occupational therapy (OT), and seven of thirteen for speech-language pathology (SLP). Mailing lists for physiatrists were obtained through provincial-territorial Colleges of Physicians and Surgeons. Total cost for purchasing rehabilitation provider mailing lists was $9,348.88. The pool from which the sample was drawn for rehabilitation providers was 22,291. Over 30% of names and addresses in the final sample pool were added through provincial–territorial regulatory body mailing lists (37.3%, 24.5% and 36.2% derived from OT, PT, and SLP, respectively). For HIV service providers, mailing lists were obtained from HIV and/or national professional organizations, web sites, Colleges of Physicians and Surgeons, and snowball sampling. The sample pool for HIV specialists was 819. Snowball sampling contributed to 6% of the total HIV sample.

Conclusions: Cross-checking national professional organization lists with those of regulatory bodies increased the number of rehabilitation professionals in the sampling frame. Snowball sampling is useful in obtaining mailing list information for some HIV service providers. This method may become more commonly used when acquiring mailing lists for health care providers in future survey research given recent implementation of privacy legislation.

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POTENTIAL COST EFFECTIVENESS OF AGGRESSIVELY TREATING MULTI-DRUG-EXPERIENCED HIV-POSITIVE PATIENTS

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Plain Language Summary: We examined what combination of efficacy and toxicity would be necessary for a "Mega-HAART" regimen to be cost-effective. The most important determinants was a low toxicity burden. Antiretroviral therapy prices may have to fall considerably for routine use of aggressive therapy to be economically attractive.

Objectives: The optimal management of multi-drug experienced HIV infected patients is unknown. We evaluated the potential cost effectiveness of aggressive treatment for this group with multiple antiretroviral drugs or novel therapies that are expensive or toxic. This approach may enhance the probability of achieving virologic suppression but could increase costs and decrease quality of life.

Methods: We developed a Markov analysis model of HIV infection to evaluate standard and aggressive therapy. The model follows individuals from the time antiretroviral therapy is initiated until death. The base case had a viral load of 40,000 copies/mL and a CD4 count of 350 cells/mm3. We assumed that individuals would change regimens after intolerance, virologic rebound, or clinical disease progression and used third line regimen. We modeled standard therapy as a 4-drug fourth line regimen and aggressive therapy as fourth line regimen a combination of at least 4 drugs but with increased costs. Patients intolerant of aggressive therapy would step down to standard therapy. Subsequently, patients started therapy which diminishes, but does not suppress, viral load levels. We modeled the effects of aggressive therapy on the enhanced probability (odds ratio) of virologic suppression and incremental costs, with base case values of 3 and $15,000, respectively. In sensitivity analyses, we increased the risk of drug-limiting intolerance to 50%, and decreased quality of life (QOL) by 10% for aggressive therapy.

Results: Aggressive therapy was associated with incremental survival of 6.4 months, discounted quality-adjusted survival of 5.3 months, and a cost effectiveness ratio of $75,500 per quality adjusted life year (QALY), if drug tolerance and QOL were similar to standard therapy. The most important determinants of cost effectiveness were the efficacy and cost of aggressive therapy; incremental costs of aggressive therapy would have to be at most $6000, $8000, or $9000 if the odds ratio for viral suppression was 2, 3, or 4 compared to standard therapy at a cost effectiveness threshold of $50,000/QALY. Incorporating intolerance and quality of life effects, the maximal incremental cost was $4000.

Conclusions: Our model suggests that aggressive HIV therapy may be cost effective if adverse effects are minimal. Ongoing trials may characterize the clinical parameters necessary for cost effectiveness, but antiretroviral therapy prices may have to fall considerably for routine use of aggressive therapy to be economically attractive.

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