F I A R

The Foundation for Integrative AIDS Research

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The12th Conference on Retroviruses and Opportunistic Infections (CROI): FEB, 2005

Brief Report

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Note: *There have been several reviews of this conference by many excellent publications. There are 967 abstracts which can be reviewed on the internet at http://www.retroconference.org. Note that, especially if you have access to a high-speed internet connection, you can view many of the sessions as they occurred. Audio is also available as are the slides. See also the list of other organizations at the end of this article for other reviews of the conference.*

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Introduction and How I Spent My Time…

As each year goes by, the increments of new knowledge remain modest. Like the quiet before the storm, we can hope these many small steps will coalesce synergistically to produce the major breakthroughs. Still, there were many nuggets of useful information that may help people in making treatment decisions--and some increased hope that more people worldwide with HIV will have the opportunity to make such decisions! The abstracts and many of the sessions may be viewed at www.retroconference.org.

There is still a woefully long way to go to assuring access to antiretrovirals (ARV) in developing nations considering the vast resources and talent that should yet be bent toward this task. This situation is unconscionable given the years of broken pledges and promises and the millions of men, women and children who have suffered and often died needlessly. While it is a depressing situation in many respects, I credit the CROI for keeping the issue of access to care, treatment and diagnostics in the developing world a central one.

During the conference, I attended all the plenaries as well as many oral and poster sessions. The days start early in the morning and usually finish fairly late at night. I also met with many old friends among the activist, physican and research communities. And I made some new acquaintances that had their own, unique insights into the mosaic of problems that HIV/AIDS represents.

In addition, I again had the opportunity to work with Fred Schaich of the International Foundation for Alternative Research on AIDS (IFARA), the stepmother of FIAR. While their mission has shifted away from evaluating "alternative" medicine, they are providing an important resource in the form of interviews with researchers at the conference. You may view these soon at the website of Jeff Palmer (whose presence we sorely missed this year) at www.pos4pos.org. They conducted some 30 interviews in English and another three in Spanish.

It was my honor to conduct three of these interviews. The first was with Jonathan Mermin from the CDC's Global AIDS Program based in Entebbe, Uganda. We discussed their approach of bringing testing and care to entire families in rural areas of Uganda. This seems like an important program that could have great benefits. He is a strong promoter of the PEPFAR program and at least in the context of families where couples may be serodiscordant (one HIV+, the other not), they can promote condom use. They are also providing some treatment.

One serious issue which I feel he downplayed was with regard to the issue of stigma and discrimination. He noted that there is a 1% rate of domestic violence. He said that after the program commenced, this increased only 1%--but that sounded to me like he meant after the institution of the program, the rate DOUBLED to 2% (which is probably already significantly under reported). If this is the case, I hope they are able to undertake appropriate steps to thwart this, lower the rate and/or find means to protect individuals from the ravages of domestic violence. The lives of millions of women and children depend on the development of robust programs. The situation, due in part to significant public awareness programs in Uganda, is probably less worse there and thus this model must be more carefully developed before it is transplanted, I believe, to other countries where denial and indifference may exist.

I then had a great interview and discussion with Jon Kaiser, MD who is based in San Francisco. He has written the book "Healing HIV Naturally" a few copies of which are available through the www.newyorkbuyersclub.org for a nominal price $3.00. Dr. Kaiser had presented a poster at the 11th CROI of a powerful antioxidant protocol. The original intent had been to see if it would affect neuropathy but the surprise was that there was a statistically significant increase of about 25% in CD4 counts in the treated group versus placebo. He anticipates that the study will be published in the summer of 2005. More information on the study is available in my report from that conference (at http://www.aidsinfonyc.org/fiar/11thCROI-fin.html) and on his website www.jonkaiser.com .

Finally, I also conducted an interview with Daniel Kuritzkes of Brigham and Women's Hospital of the Harvard Medical School in Boston. We discussed chemokine receptor inhibitors. These drugs may either target part of

HIV (as T20/Fuzeon targets gp41) or the coreceptors that T cells express, like CCR5 and CXCR4[1]. As noted, these interviews, and all the others, may be viewed on Positive for Positive's website.

Recent data underscore concerns that the use of an R5-inhibiting drug may result in the clinically more dangerous HIV variants that use the X4 receptor to predominate (#361; *JAIDS* 2005 Apr 1;38(4):382-392). In poster 361, researchers noted that people with the missing portion of CCR5 ("delta-32") were more likely to be infected by an X4 virus. The disturbing finding of the *JAIDS* article was that, despite effective ARV, some reservoir of HIV existed where a switch from R5 to X4-receptor utilizing virus emerged. In many cases, these were probably there before people started ARV, just waiting for their moment in the sun.

The Putative HIV "Superbug"

This year's major controversy centered on a potential "super strain" of HIV. A special session was set up on Thursday evening, but the mood at the Congress was decidedly cold and skeptical. As many have pointed out, rapid progression to AIDS is not new. Nor is infection with multi-drug resistant strains of HIV, which other documented cases do not suggest leads to faster progression. To put it mildly, despite the apparently unusual sequence of this virus's genome, it was felt the announcement was premature. The person that is suffering from this disease is a man in his 40s who had had frequent unprotected, receptive anal sex at sex clubs while using a crystal meth. A question not raised was to what degree meth use may accelerate disease progression or if it may have an impact on the virulence of the HIV itself (see, e.g., Beck et al., *Trends Microbiol*, 2004 Sep;12(9):417-423 where they found that nutritional deficiencies can make the virus nastier or more *virulent*).

During his presentation, Ho stated that, in addition to the unusual sequence of the HIV, they had tested the patient for immunological factors that may be associated with faster progression. In the recently published article, they note "We did not find HLA alleles that have been associated with rapid progression, including A*24, B*35 Px, B*37, B*56, B*58S, and A1-B8-DR3" (*Lancet*, 2005;365:1031-1038). They are investigating other markers associated with more rapid disease.

Other alleles that are associated with slower or faster progression have also been identified.There are recent data suggesting a strong correlation with the expression of CCL3L1 and both susceptibility to infection and progression rate (#146 and *Science*, 4 Mar 2005;307:1434-40). It is not clear if they evaluated that yet in this person. CCL3L1 is a protein that binds to CCR5. People with more copies of the gene and higher expression tend to have reduced rates of disease progression.

Still, it is also true that a particularly virulent form of HIV *may* have arisen and only time will tell. Dr. David Ho of the Aaron Diamond Institute presented the data on behalf of Marty Markowitz and the physician, Michael Mullen, who originally encountered the patient. Ho points out that if indeed the individual was infected in October and subsequently had a CD4 count around 80 by December, this would represent one of the fastest rates of progression ever recorded to date. But there is a 10-month period during which the individual had not been tested. So it is unclear exactly when the fellow was infected as his last test date was in May, 2003.

There are larger social ramifications that create a great deal of concern. On the one hand, if creating greater awareness among those for whom HIV has receded as a major concern has the effect of helping people make better choices like using condoms and adopting harm reduction strategies for drug use, all to the good. Indeed, the sky HAS been falling for several years and all the successes of antivirals don't mitigate an undiminished incidence of new infections. If, however, no greater threat materializes, it may cause people to have less trust in public health pronouncements and may lead only to a further erosion in public confidence and a further deterioration in safer sex practices and harm reduction programs.

Politically, if names reporting and mandatory testing are the policy response, it will be a great loss, both as a violation of civil rights as well as a fiscally irresponsible move. If the result is further stigma and discrimination

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[1] HIV bbinds to T-cells. These cells have proteins sticking out of their surface that allow proteins (gp120) sticking out of HIV to connect. The main protein on the T cell is called CD4 (so a T-cell expressing CD4 is CD4+). HIV also uses its gp41 protein to connect to other "co-receptors" nearby, the most common one being CCR-5 (or R5 for short). Another one that is associated with poor outcomes clinically is known as CXCR-4 (X4). These are chemokine receptors.

against gay men, this only adds fuel to the fire of the pandemic. Indeed, the characterization of gay men as drug-using sex maniacs is already being seen in the media. Premise: gay men have lots of sex. Reality: some do, some have few partners (as with women). Premise: lots of sex is bad. Reality: sex is not bad; transmitting or becoming infected is a public health problem that people should avoid. What is needed are targeted interventions, good sex education, frank discussion and easy availability of condoms, harm reduction programs, etc.

Premise: gay men do a lot of drugs (street or otherwise). Reality: There are some data suggesting higher rates of recreational drug use among gay men as a group than heterosexual men, bisexuals, lesbians, etc. Many don't use or overuse drugs. And the reality is that use of recreational drugs remains high in various populations. Indeed, a study in Australia indicated that some 39.1% of surveyed individuals had ever used recreational drugs (*Social Research Briefs*, 2004 Mar;4:1-2).

Premise: using recreational drugs is bad. Reality: some people can enjoy them safely or with reduced harm. The reality is that many substances are used by people either for pleasure or to reduce pain. These include alcohol (which in small quantities can be healthful), tobacco, heroin, antidepressants, amphetamines, LSD, fatty foods. (Fatty foods—a recreational drug?) Can any of these cause harm? Of course. And some may accelerate HIV disease progression. Others, like marijuana, may be helpful as a medicine to increase appetite and reduce drug-associated nausea. Does it make sense to make some illegal while others—potentially even more harmful such as tobacco—are not? No. Not from an ethical sense of incarcerating people (more often people of color and those in the lower socioeconomic strata: racist and class war). Nor from a fiscal standpoint (see the review from Rand Corporation[2]).

Addressing public policy aspects of recreational drug use means that better solutions need to be developed than the punitive "war on some drugs" that has been SUCH a dismal failure. Indeed, rather than moving to a more rational public policy toward substance use, the Bush administration and their cohorts in Congress are carrying on a direct assault upon the very programs that are making a difference and a significant dent, to wit, the harm reduction programs.

Premise: people who have unprotected sex deserve what they get. Reality: NO ONE deserves infections or disease. Not the perfect and chaste, not the smoker, indolent, drinker or obese. The issues to address are ignorance, sadness/grief, discrimination, stigma, powerful human emotions and drives.

The "supervirus" controversy stands in stark contrast to what I felt was a more legitimate issue raised at last year's conference: the outrageous and vicious increase in the price of ritonavir (Norvir). The overall battle was lost in that the price remains outrageously high. However, many other battles along the way were won and a significant and decided strategic development has arisen that has galvanized physicians who were rightly furious and worked together in a hitherto unheard of fashion to bring pressure and their outrage to Abbott's attention. State AIDS Drug Assistance Programs (ADAPs) were preserved from this price hike. And a new uneasiness was instilled in the industry that its outright rape of patients will not be tolerated. Let's not forget that Abbott's extreme greed does not in any way mean one can construe prices for MOST pharmaceutical prescriptions, particularly ARV, as being "reasonable." Drug pricing is out of control in the United States—and the industry is doing its level best to assure that this assault on health persists by hiding behind monopolistic use of patent law is a global phenomenon.

As the current administration's lies and failures mount, a new wave of political reality will arise that will of necessity result in more rigorous legislation, including ultimately price controls and a robust single payer healthcare system. This is not only humane and ethical, it is also fiscally responsible when 15% of the GDP--and some estimate that this figure will rise to nearly 20% in a few years--is spent on healthcare costs which are steadily rising faster than the rate of inflation year-over-year while over 45 million U.S. citizens are uninsured. Meanwhile, those with insurance are seeing rapidly rising co-pays, premiums, loss of work-related health care while the patchwork system collapses under the weight of the industry's extreme greed. Not only with regard to drug prices, but also diagnostics, devices, reagents, licensing fees and the myriad costs (induced often by bizarre distortions and abuse of intellectual property and patent law) that stymie research, narrow the new drug pipeline and create enormous suffering and death for millions domestically and internationally.

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[2] http://www.rand.org/pubs/occasional_papers/2005/RAND_OP121.pdf.

The "Cured" Guy…

At the other end of the spectrum and with virtually no fanfare was a curious abstract about the experience of a fellow in Israel who had a documented HIV infection, with increasing viral load and declining CD4 count (poster #310). He was place on ARV for a time and eventually stopped after his CD4 count increased substantially well into the normal range. Unlike most people, he remained with an undetectable viral load even after stopping ARV (not unlike the "Berlin patient"). Due to the sustained undetectability of the HIV, the group, run by respected immunologist, Zvi Grossman, ran another HIV test (ELISA, Western blot) and after four years, he was HIV-negative.

Such seroreversions are rare but have been documented, according to the researchers. Generally, it is seen in infants who lose maternal antibodies to HIV but haven't actually been productively infected. The difference here was the length of time from documented seroconversion to the observed seroreversion (four years). Generally, seroreversion to HIV-negative occurs within a few weeks of infection (see, e.g., *Clin Infect Dis*. 2005 Mar 15;40(6):868-73). In addition, as they note, "rare cases of HIV-1 seroreversion have been reported for patients with advanced or rapidly progressive disease."

The questions that arise include why the immune system would stop generating antibodies to HIV? Many viral infections result in a persistent expression of antibodies that may last through life. Is this a sign that this individual did not have an excessive immune response (like sooty mangabeys)? What are the implications for vaccine design?

"CAM" Therapy[3]

Believe it or not, there was actually an oral presentation regarding the use of fish oils in the management of hypertriglyceridemia (#39). The study used MaxEPA, which contains fish oil composed of different omega-3 fatty acids. These include DHA (12%) and EPA (18%). A review of essential fatty acids may be found at http://www.aidsinfonyc.org/fiar/EFAs.html.

The study was conducted in France and was a double-blind, placebo-controlled and randomized study of 122 people over 8 weeks. Before the 8 weeks of the trial, participants receive four weeks of an appropriate diet as part of the management strategy. At the beginning of the 8-week phase of the study, people were randomized to receive either fish oils or placebo capsules (paraffin oil). Participants received 1 gram capsules of the fish oil and they took two, three times per day. Then, at the end of the 8-week study period, all patients received MaxEPA for an additional 8 weeks for an open-label phase of the study.

The results were "In an intent-to-treat analysis, median change of triglycerides was –25.5% in group M [MaxEPA], vs +1% in group P [placebo] at week 8 ($p = 0.0033$)." In other words, the interventions, despite a modest daily dose of 6 grams a day, showed a substantial reduction in the triglyceride level. This is almost as good as a *combination* of fluvastatin and triglyceride-lowering fenofibrate, which was about 33% (*Clin Ther*. 2004 Oct;26(10):1599-1607.).

Approved ARV News

A number of studies were reported on that clarified ways to mix and match the currently approved medications among different populations. Populations studied varied from early disease to people with AIDS and fewer therapeutic options. Other studies, some reviewed here, looked at the effects of ARV approaches for pregnant women and impact on infants.

Good news came from a study that compared the regular dose of d4T (stavudine/Zerit) of 40 mg twice a day to using only 30 mg twice a day (#857). They found similar success rates for both doses in terms of virological response (driving the load to below 400 copies). The lower dose, as you might expect, resulted in significantly better outcomes in terms of lower triglycerides and total cholesterol, as well as increases in limb fat. They also

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[3] CAM="Complementary and Alternative Medicine" - the stuff that is "non-allopathic." This is essentially an artificial distinction. If it's good medicine, it alleviates or cures disease. The distinction is often one that is more political than genuine, I believe. Intellectual property rights and out-of-control drug pricing drive the distinction, in part, as most CAM are not patentable.

studied a group that switched to tenofovir and improvements in these parameters was even stronger. But for those who need to use d4T due to lack of other options or other reasons (or access only to generics), lower doses with equivalent antiviral efficacy and less toxicity is an extremely important finding. Could 20 mg bid work as well?

A study (#578) conducted in France looked at a group of 82 people who had resistance to nukes. They gave them two boosted protease inhibitors, including saquinavir-lopinavir-ritonavir, indinavir-lopinavir-ritonavir or indinavir-amprenavir-ritonavir. This last combination appeared to produce the best results in terms of achieving an undetectable viral load below 400 copies (76%, 84% and 93%, respectively). Gastrointestinal trouble was the reason some 20 participants switched to another PI. That the indinavir-amprenavir-ritonavir combo was most successful is bad news only in the sense that for many, the outrageous cost of ritonavir not used as Kaletra (lopinavir-ritonavir) may make it unattainable. Thank you, Abbott, for helping increase suffering and death because of your greed.

Use of ARV among pregnant women also received further scrutiny in presentation papers 134-140. A study in Kenya showed that 15% of women with under 250 CD4 cells and 7% of those with higher CD4 counts experienced toxicities related to nevirapine, including rash, liver toxicity, neutropenia or sometimes a combination of these (#809). While not so helpful where protease inhibitors are unavailable or too costly, a study showed that women who used them had much less severe reactions than were seen when nevirapine was used (#784). Some good news was seen among women with more than one pregnancy where transmission to the infant did not increase with subsequent pregnancies and, despite starting and stopping ARV, control of the virus actually improved between the first and second pregnancy (#786).

Other investigators found that among 97 women with higher T-cell counts (median 496) in the Ivory Coast, 53 births did not result in infection despite no treatment. However, women with lower T-cell counts saw significantly reduced rates of transmission, especially if they received AZT+3TC+nevirapine (instead of just NVP or AZT+3TC) during the third trimester (week 28). However, there were 3 stillbirths in the cohort and 6 of the women on the stronger combination had to change ARV due to a grade 3 adverse event (rash, liver toxicity or anemia) (#785).

Several papers reviewed effects of treatment on uninfected infants. In one (#803), they compared 6 weeks of AZT to nevirapine monotherapy in South Africa. Of 718 infants they presented data on, 570 (79%) were exclusively bottle-fed. By 100 days of age, there were 116 hospitalizations, 28.3% of them HIV-infected and 13.3% not. Deaths were seen in 6.9% and 1.3% of HIV+ and uninfected infants, respectively. After analysis of the data, what they found was that the mother's health (e.g., viral load) had the most impact on infant survival. By contrast, the method she used to feed the infant did not have an impact. The data clearly indicate that preserving the mother's health will greatly improve that of the child. Treat the mothers!

Another study tended to confirm this (#804). The kids born without HIV were more likely to suffer if the mother's CD4 count was low. The researchers hypothesize that the lack of transfer of maternal antibodies to the infant results in an increased risk of infections among the infants. Kids need the mother's immunity in the first months to help fend off infections. In Zambia (#805), they observed a 4.9% mortality rate among HIV-negative infants by 4 months. Morbidity (illness) and mortality (death) were a greater risk for infants if the mother's CD4 count was below 350. Nutritional deficiencies, as well as immunological, were identified as potential culprits.

New ARV News

Good reviews: http://www.natap.org/2005/CROI/croi_42.htm, http://www.catie.ca/aidsinfo.nsf/news

The RESIST study showed ritonavir-boosted tipranavir worked significantly better than boosted lopinavir (trade name, Kaletra) among PI-experienced individuals (#560). This was a fairly good sized study with 1,483 people randomized over 24 weeks. Of course, 48-week data are better for assessing the durability of this effect. Tipranavir continues to show potential for PI-experienced people.

The new Tibotec drugs are ones to keep an eye on. Further data on their protease inhibitor, known as TMC114, was presented (abstract 164LB) underscoring a substantial 1.3 to 1.85 log drop in viral load, depending on the dose taken. Each dose was boosted with 100 mg of ritonavir. The best dose appeared to be 600 mg TMC/100 mg

ritonavir taken twice daily over the 14 days of the trial. This resulted in a mean increase of 75 T cells. It appears to be effective even in the presence of resistance to other members of the class, as the study was among people who had experience with one or more protease inhibitors and had genotypic and phenotypic evidence of resistance. Nearly half the participants were also taking T20.

One note of concern regarding the way the data were presented. It seems there were 4 arms (differing doses in each arm) meaning that 600 people were enrolled. Data were provided only on 494 patients, however, although they indicate that the analysis undertaken was the more rigorous "intent-to-treat" which includes people who may have discontinued due to virological failure or adverse events. What happened to the other 106 patients? Further, the abstract does not specify the nature of the grade 3/4 adverse events, which were experienced by 26% of participants over most of the arms. "Serious" AEs ranged from 9-17% although the lower percentage was seen in the highest dosed arm (though the differences were not statistically significant between arms). Is “serious” the same as exclusively grade 4 toxicity?

Another drug from this company, TMC125-C201 (also known as TMC278) is an NNRTI update on a predecessor drug. It is a diarylpyrimidine and was evaluated as monotherapy over 7 days. Headache was the most frequently reported side effect. All doses achieved around 1.1 to 1.3 log drop in HIV load (suggesting a twice per day dose of 25 mg is adequate).

Also, a derivative of the triterpene, betulinic acid, known as PA-457, (#551), operates on the primary protein that makes up the capsid (p24). The capsid protects HIV's RNA. Thus, with the inhibitor present, the virus can't process the p25 form into the p24 form with the result that the viruses that bud out are not infectious and remain immature. This class of drugs is being referred to as maturation inhibitors. (Other data have underscored a potential second mechanism via binding to HIV's gp41 envelope protein as T20 or enfuvirtide also does.)

There were two studies. The first was undertaken over 10 days among HIV-negative volunteers. The drug was found to be safe and well tolerated even at high doses, although even the lowest dose of 25 mg once a day achieved blood levels sufficient to knock out 90% of the virus (IC_{90}). The other report (#159) was a double-blind, placebo-controlled study of a singled dose of 75, 150 or 250 mg among 24 HIV+ people with CD4 > 200 and a viral load between 5,000 and 250,000. Viral load dropped in a range from 0.17 (in the placebo group) to 0.5 log in the highest dose. It also appeared to work in people with resistance mutations in the HIV that reduce the benefit of other ARV drugs. Longer studies are in development. What's a little frustrating is that this product had been discussed in 2003 at the 10th CROI. The development seems to be achingly slow.

Betulinic acid (BA) is found in several plant species. Whether BA itself can achieve these effects is not certain, however perhaps FIAR will be able to fund a study one day of *Cynomorium songaricum*, Rupr., which Huang notes is used to improve body immunity and stimulate the endocrine system. Also, the leaves of *Syzigium claviforum* have considerable amounts of betulinic acid. RPR 103611 is another betulinic acid derivative that has been under investigation at least since 1993 when GMHC’s *Treatment Issues*, vol. 7, no. 7 of July/August, 1993 reported on test tube data for the drug. They note also that it is found in the root of *Tripterygium wilfordii*.

Amdoxovir (DAPD) is a nuke that was tested in people for whom current treatments are generally failing to exert virological control (#553). Participants were on an optimized background therapy consisting of 3-5 drugs, depending on genotypic/phenotypic testing at screening and were also on T20 (the injectable fusion inhibitor, Fuzeon). They then either received 300 mg of drug twice a day or a matching placebo. At the end of the day, there were no statistically significant differences between placebo recipients and drug recipients according to viral load (-1.1 log versus -0.8 log in the placebo arm) or CD4 count (70 vs. 54 more T cells).

A new potential class of drugs targets a cellular protein that the HIV protein rev uses to enhance HIV replication in infected cells (#548). The protein targeted is known as deoxyhypusine hydroxylase (DOHH) which in turn enzymatically activates another protein, known as eukaryotic initiation of translation factor 5A (eIF5A). The two drugs they tested were a topical antifungal, known as ciclopirox and a drug used to treat iron overload, deferiprone. Ciclopirox is used to treat a fungal infection of the nails called onychomycosis. One proposed mechanism of action for this drug is also one that acts as an iron and aluminum chelator.

Yet again, though, this isn't exactly new and was described as early as 1998 (see *Biochem Pharmacol*. 1998 Jun 1;55(11):1807-18). Why haven't they been studied clinically? (Is it too cynical to think it's because they're not patentable?) In the *Biochem Pharmacol*. study, they also looked at a natural product and also an iron chelator known as mimosine, "a non-protein amino acid found in leaves, pods and seeds of tropical legumes of the genus *Leucaena*."[4] Mimosine also arrests the cell cycle at the G1 phase. These drugs may have application in cancer treatment, as well as the grape-skin extract, resveratrol, aside from their potential as anti-HIV therapies.

Pathogenesis News

Understanding how HIV infection results in the development of AIDS is critical to our better understanding of how to manage the disease. This is known as *pathogenesis*. Part of that is understanding where the virus hides. We know it likes to hang out in T cells (CD4+ T lymphocytes), preferably ones with a resting memory phenotype but also activated cells as well. HIV also infects other cells (like macrophages, dendritic cells, etc.) We also know that HIV winds up in the lymph system as a result.

Danny Douek gave a plenary lecture (session 33, poster 127) on recent evaluations of the gut of people who had just been infected. They found that the acute infection was associated with a massive disruption and depletion of the lymph tissue in the gut: HIV is a disease of the Gut! (Well, OK, we've known that for well over 10 years but this was more direct evidence).

Douek's presentation underscored the very powerful interplay between direct activity of HIV destroying the very CD4+ T cells that could fight it along with host immune and inflammatory damage contributing to further CD4 losses. The seeding of HIV to the lymph nodes (and other organs) then results in further disruption and destruction of bystander (uninfected) CD4 cells. The architecture of these lymphoid organs is a key feature to understanding how immunity is compromised so severely and results ultimately in the development of AIDS.

There is still no consensus on what the implications are therapeutically. There are some data emerging to suggest that the use of a combination of antiretrovirals within a week or two of infection may well help minimize the gut damage and reduce the viral "setpoint" which may ultimately slow disease progression. That particular study also suggested that starting ARV treatment after a delay of more than a few weeks probably will have little additional benefit in terms of effects on viral setpoint or progression rate. Is there any recovery of gut mucosa once infection is established? Are there therapeutic interventions that may help to accomplish that? Might gut-assisting interventions ranging from glutamine to acidophilus/bifidus/*S. boulardii* be of benefit? And, of course, as Douek pointed out, these direct effects of CD4 destruction by HIV and subsequent dissemination of the virus may initiate but not be the direct cause of the death of bystander CD4+ cells, the majority of which that die by apoptosis (cell suicide) in chronic HIV disease, most of which are <u>not</u> infected.

In past CROIs, there was a great deal of discussion about a pool of stable, latent, memory CD4+ cells (T cells come in different varieties) as a sanctuary site for HIV to hang out, even during the monsoon season of ARV therapy. Are there other sites? One group looked at 10 HIV+ individuals on ARV who bravely gave up to intense sampling over a 3-month period, providing samples every 2-3 days (#304). Their viral loads were below 50 copies. The researchers then analyzed the sequences of the HIV in different cells, particularly looking at these resting memory cells and at the fluid cells reside in, the plasma. Using ultra sensitive PCR techniques, they evaluated sequences of the *pol* gene of HIV (which encodes for reverse transcriptase). What they found was disturbing. Most had pretty much the same sequences in the resting cells as in plasma. But about a third of patients had some totally different sequences.

This brought them to two conclusions: "First, a second major reservoir contributes to persistent viremia in some patients on HAART. Second, the latent reservoir in resting $CD4^+$ T cells is not maintained by ongoing viral replication as its composition does not reflect that of the low-level plasma virus in some patients. Thus the reservoir is intrinsically stable, consistent with the biology of memory $CD4^+$ T cells, and is unlikely to decay even if low-level viremia is further reduced by intensification of HAART."

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[4] http://www.ansci.cornell.edu/plants/toxicagents/aminoacid/mimosine.html

Two articles from HIVandHepatitis.com nicely covered the issue of why sooty mangabeys don't develop AIDS and studies presented at CROI that endeavor to answer that question. The second article deals with different cellular cofactors that HIV uses to replicate. See the Appendix for the full articles.

Drug Side Effects and Complications

Heart trouble

Review of ARV side effects have become a key feature of CROI. Wafaa el Sadr gave a rather devastating review of the D:A:D study which evaluated cardiovascular effects (#42 and #866). The short story: for each year of combination antiretroviral therapy (cART), there is a 17% increased risk of myocardial infarction[5] (MI; a heart attack). This is substantial and serious.

These data are derived from an evaluation of 23,441 HIV+ people from 11 cohorts in Europe, Australia and the U.S. They found that the relative risk was a whopping 4.38 for those on ARV for 6 years or more. The overall risk for a first MI was 17% (as noted) and for a second MI the same: 18%. While older age is an increased risk factor, they were seeing the high risk among younger individuals too. Improving the lipid profile markedly reduced MI risk—but did not eliminate it. This may be due to the damage to heart vessels through mitochondrial toxicity, underscoring the potential for antioxidant therapy in addition to lipid lowering approaches to further reduce the MI risk. The idea that antioxidants (NAC, alpha lipoic, B vitamins) may reduce oxidative stress and subsequent risk of an MI, though, remains speculative.

One study (#859) showed that a switch of ARV was not necessarily adequate to improve lipid profiles. Interventions such as statin therapy may be needed. By contrast, some may benefit from other approaches, such as the use of fish oils, sugarcane wax-derived policosanols, pantethine and so forth.

Liver trouble

For those with access to ARV, the chance of dying from an OI has been substantially reduced. Overall mortality has been dramatically reduced with nearly two million years of life saved so far (#143LB). But people are still dying and one of the most common reasons for death is due to liver failure.

Most commonly, this is associated with chronic infection with either hepatitis B or C. One study suggested a more rapid progression among even people who had an initial biopsy that showed mild liver disease among HIV/HCV co-infected people (#121). Nearly 25% advanced from mild to significant fibrosis (tissue scarring) over 2.84 years (median) between biopsies. Would milk thistle help to slow this down?

And, of course, the liver toxic effects of some of the ARV, especially ritonavir (Norvir) and nevirapine (Viramune). A couple of studies (#832, 833) noted that nevirapine and efavirenz are both metabolized by the liver by an enzyme known as cytochrome P450 2B6. One mutation found in some people seemed to protect against liver toxicities (C3435T). People lacking this though were more likely to see liver toxicities and in #833 they noted that some 17% (66 of 385) of South African adults experienced grade 3/4 liver function test elevations related to drug.

One bit of good news was reflected in paper #120 where more evidence was provided for methods to evaluate the state of the liver by bloodwork. Up until now, the use of a biopsy to extract a sample of liver has been the primary means for assessing the stage of liver disease. In this presentation, they note that one may use a simple equation to evaluate the level of platelets to determine whether there is early, mild disease or late disease or cirrhosis. It tends to not work as well for people in the middle stages. There are other markers that are fairly routine, such as haptoglobin, which are being used in Europe and elsewhere (Fibrotest) to assess liver function. It is to be hoped that the costly and invasive biopsy procedure will soon be largely a thing of the past.

Treatment has continued to show a reduced benefit for the use of pegylated interferon plus ribavirin among co-infected folks compared to mono-infected. Taking AZT in the mix is indeed associated with more severe anemia, which either ribavirin dose reductions or the use of drugs like erythropoietin (Procrit, Epogen) does not

[5] An infarction is damage to tissue caused by a loss of blood flow. Myocardial means heart muscle. So an obstruction in one of the arteries supplying blood causes damage to the muscle tissues.

necessarily compensate for (#477). Maintaining an adequate level of ribavirin is important to enhance the likelihood of a sustained response—that is, undetectable Hepatitis C levels 6 months after stopping treatment. The minimum level appears to be 1 microgram/milliliter (#928). On the other end, keeping ribavirin blood levels below 2.7 micrograms/ml helps to prevent ribavirin-associated anemia (#929).

Given the severity and dangers of liver toxicity, FIAR is delighted to be working with Mount Sinai Medical Center and Drs. Henry Sack, Doug Dieterich, Richard Mackay and the team on a study evaluating the safety and efficacy of the botanical, *Silybum marianum*, also known as Milk Thistle. This is a year-long study, double-blind, randomized and placebo-controlled. To learn more, see: http://www.aidsinfonyc.org/fiar/mt.html and our website at http://www.aidsinfonyc.org/fiar .

Of course, there are a variety of other agents and formulas that have shown varying degrees of benefit in slowing disease and restoring liver function. Among these are antioxidants such as N-acetylcysteine (NAC), alpha lipoic acid, whey proteins, and botanicals such as artichoke, glycyrrhizin (from the licorice root of *Glycyrrhiza glabra*) and combinations such as the Chinese formulas known as Ecliptex, Hepato-C and Sho Saiko To. Further clinical studies in the setting of chronic HIV infection with concomitant hepatitis B and/or C are critical. It is frustrating that so few studies have begun of these agents.

Structured Treatment Interruptions (STIs)

A variety of studies were reported on that evaluated various scenarios under which people may stop their ARV regimen Reasons for doing so include having a high CD4 count with virological control due to ARV, toxicities and/or failing regimens. Methods of stopping include fixed periods of time off drugs (which overall don't seem to work as well) or CD4-guided approaches where ARV is resumed once the CD4 count drops to a threshold level. The latter method, of course, is applicable only among those who have higher CD4 counts (e.g., above 350).

Results from CPCRA 064 evaluated individuals who opted to stop their regimen with a lower CD4 count (#579). Here, ARV-experienced participants with evidence of multi-drug resistant viruses were randomized to either a four-month STI followed by a new regimen or an immediate switch. The results were not good for those taking the STI. They saw no benefit in terms of clinical, immunologic or virologic outcomes--nor even in any improved quality of life. Indeed, overall, their CD4 responses to optimized ARV after the interruption were not as robust as those who had an immediate switch.

This raises an issue as to whether some OTHER type of intervention during the STI might not improve outcomes. FIAR members Mark Kuebel, Lic. Ac. and Fred Blair, Lic. Ac. have been developing a Chinese formula designed to improve outcomes and extend the time on an STI. Originally planned for those with higher CD4 counts, several individuals with low CD4 counts and on ineffective ARV regimens have tried the regimen. Two appear to remain clinically stable. No conclusions whatsoever may be drawn from these anecdotes, however we hope to fund a somewhat larger pilot, open label study to evaluate the regimen.

In poster 679, the Tibet study was discussed. Here they sought to observe the evolution of drug resistant variants of HIV during an STI. Overall, about 36% of patients had some detectable resistant viruses. What they observed was that for NRTIs and PIs, pre-existing virus that had some resistance grew out, rather than being selected as a result of viral evolution. However, people on NNRTIs did see new viruses emerging that were resistant in some 42% (8 of 19) of the patients who were using NNRTIs in either the first or second treatment interruption. The authors suggested that people using NNRTIs not undergo treatment interruptions.

Another presentation (#681) underscored that the numbers of ARV-resistant virus begin to decline during an STI. This indicates a fitness cost to the virus to be able to replicate under the pressure of ARV therapy and the wild-type begins to recover. They noticed that major, minor and novel mutations disappeared during an STI and think that this knowledge may help to design clinical strategies to further cripple HIV and reduce its fitness.

It is my thought that perhaps people probably did not stop the NNRTI first, while continuing their other drugs for at least a few days to up to a week or two before discontinuing the rest of the regimen. This is due to the long half-life of drugs like nevirapine and efavirenz. If all the drugs are stopped at the same time, the NNRTI drugs

will still be in the body, effectively resulting in a monotherapy treatment and thus permitting the emergence of HIV resistant variants.

Other studies have shown differences along gender and ethnic lines that may further complicate the issue. For example, women appear to have residual efavirenz levels in their blood for up to two weeks after stopping the drug (11th CROI). Others have noted that nevirapine (Viramune) use among women with a low body mass index should be discouraged due to liver toxicities (*J Infect Dis*. 2005 Mar 15;191(6):825-9). Others have noted that "Patients from South America and Western countries had higher clearance of nevirapine compared with Thai and South African patients" (*Antivir Ther*. 2005;10(1):145-55). African-Americans are more likely to have a particular host gene (CYP2B6 T/T genotype, G516H) that may result in efavirenz sticking around in the body longer and may also be associated with central nervous system reactions.

However, whether stopping the NNRTI first and subsequently stopping other ARV would prevent resistance from developing has not been studied. Nor is it clear how long people should wait before stopping their other ARV drugs. Two-three days? Two weeks? Such concerns must be carefully weighed for those considering a treatment interruption who are using an NNRTI.

For people with higher CD4 counts, STIs may remain a reasonable option. One approach sought to maximize benefit through the use of a therapeutic vaccine along with administration of IL-2 (#133LB). They looked at patients with a viral load below 50 and CD4 counts above 350. During ARV treatment, they were randomized to ARV alone (37) or given 4.5 million units of IL2 twice a day, subcutaneously for 5 days along with two vaccines, ALVAC vCP1433 and HIV-Lipo-6T (34 patients). After 40 weeks, they started an STI. They remained off of ARV as long as the viral load stayed below 50,000 at week four and subsequently below 10,000. If they started back and achieved a load below 50, they commenced another treatment interruption and follow-up. All but one completed the study. Median time off treatment was 177 days for those on the combined treatment, significantly longer than the 89 days for those who did not receive the IL2 and vaccine treatments. The group went through three treatment interruptions. Those without vaccine+IL2 had higher viremia at the end of 4 weeks of each STI (4.81, 4.44, 4.53 log10) than those with the enhanced treatment (4.48, 4.00, 3.66). It was interesting to see that viral rebound was somewhat attenuated in the combined treatment group. They noted that "these effects translated into a significant reduction in exposure to HAART."

Another study looked at IL2 (#582) among 47 people with CD4 counts above 500 and viral load below 200. They were randomized to either a set of three, 5-day cycles of IL2 (4.5 MiU, bid, subcutaneously) or to just receive ARV. They then started an STI and stayed off ARV until the CD4 count fell below 350. As you might expect, the IL2 group had more CD4 cells than those that just received ARV, but this difference waned after 20 months. One important element of how quickly people may have had to restart their ARV was their lowest-ever CD4 count (the *nadir*), as well as their viral load. Those who had had a high viral load or a lower CD4 nadir were more likely to return to ARV sooner.

See also Treatment interruptions may last longer with interleukin-2, *CATIE-News* at http://www.catie.ca/aidsinfo.nsf/news where their excellent report notes: **"**Regulatory authorities in the European Union have approved the testing of IL-2 as a mist which is inhaled and goes to the lungs of HIV negative patients with kidney cancer. Researchers hope that by sending IL-2 to mucosal tissue in the lungs the drug will be better tolerated and the immune system will be stimulated to control kidney tumours."

Developing Countries and Access

As has now become a routine and important aspect of CROI, the issues of access took center stage. The news is still grim as too many millions are suffering and dying needlessly, but for transnational corporate greed, corruption and the cruel and misguided efforts of the Bush administration to derail the Global Fund by setting up the PEPFAR program.

The problems with PEPFAR include:
The emphasis on "abstinence only" monies;
Utilization of the Gag Rule to inhibit family planning;

Funding of fundamentalist religious organizations (exclusively Christian?);
Turning the FDA into the regulatory agency for the planet by requiring approval by them of generics;
Destabilization of the UN Global Fund for AIDS, TB and Malaria (GFATM).

In terms of the GFATM, a lot of grants have been made and there is money out there but a lot of it doesn't seem to be getting to the ground in terms of people getting treatment. This is unconscionable, even while they desperately do need more funding to continue the work—it will be a farce if the money that IS available fails to achieve it's potential, people continue to suffer and die and Bush and his colleagues gain more fuel in their ongoing and direct campaign to destabilize, discredit and destroy the United Nations (as is no more clearly reflected in the appointment of John Bolton to be the US Ambassador to the UN).

Still, progress is being made and we can hope that these parallel streams will begin to intersect in constructive ways and offset their respective weaknesses. As activists, I think we can use the opportunity that these separate streams of funding represent to enhance infrastructure development and access.

Terminology

If you find some of the terms in this document confusing, please don't hesitate to inquire or look them up at: http://www.niaid.nih.gov/factsheets/GLOSSARY.htm

APPENDIX A

Viral and Cellular Determinants of HIV Pathogenesis

HIVandHepatitis.com (2/28/05) Bailey L
[Report from the *12th Conference on Retroviruses and Opportunistic Infections*]

Friday morning's session on Viral and Cellular Determinants of Pathogenesis contained 11 presentations. One theme found in six of these presentations was looking at how sooty mangabeys and African green monkeys have co-existed with SIV for millennia. These animals do not develop clinical immune suppression, and are considered "non-progressing" hosts. The sooty mangabey, like rhesus monkeys, are able to be infected with SIV and high rates of infection are seen once the animal reaches sexual maturity. But, unlike humans and rhesus monkeys, the sooty mangabey has infection that is characterized by near normal CD4 T-cell counts and lack of progression to an AIDS-like syndrome, despite high viremia. One presentation that sought to look into the reasons for this divergent disease outcome was presented by Silvija Staprans from Emory University. Her group hypothesized that lack of generalized immune activation, like that commonly seen after HIV infection, could explain this lack of disease progression. In their research, they noted that there was little, if any, NK and CD8 T-cell activation as a response to SIV infection. They hypothesized that lack of DC cell activation and lack of type I IFN production in SIV infected mangabeys may lead to an anti-inflammatory immune response that protects the host from the "bystander" damage seen in pathogenic primate lentivirus infections.

Another presentation by Ronald Veazey from Tulane University's Primate Research Center examined a factor that has been determined to be important in human HIV infection – infection and rapid depletion of CD4 T-cells in the gut. There are an abundance of activated CD4 T-cells residing in the gut. He termed these cells "fuel cells" and described these cells as being a major site of viral replication and T-cell depletion. In his study, he compared percentages of these intestinal "fuel cells" in the gut lymphoid tissue of progressing (rhesus monkey) and non-progressing (mangabeys and African green monkeys) hosts. They found that like in humans, macaques and rhesus monkeys have large numbers of effector memory CD4 T cells in their intestinal lymphoid tissue. In progressing hosts, these cells are selected depleted by SIV and HIV infection, and turnover of these cells serves as "fuel" for active viral replication. Remarkably, they found that these cells are essentially absent from infected sooty mangabeys and African green monkeys. His group hypothesized that co-evolution of these natural hosts over millennia has resulted in the selection of animals that have few intestinal CD4 T-cells (African green monkey) or that do not express CCR5 on their intestinal CD4 T-cells (sooty mangabey).

Two studies had conflicting outcomes in explaining how SIV-infected sooty mangabeys control SIV viral replication. A group presenting from Harvard University did work to suggest that SIV-specific CD8 responses were responsible in limiting viral replication during the course of natural SIV infection. However, a group from Emory University started with a hypothesis that CD8 T-cell responses are important in determining the absence of clinically relevant immunodeficiency in mangabeys, but their work in measuring the magnitude and breadth of SIV-specific CD8 T-cell mediated responses indicated otherwise. In 74 naturally infected mangabeys, they were able to measure only limited CD8 T-cell mediated responses in the majority of the animals (i.e. <0.2% of total CD8 T-cells). In their conclusion they hypothesized that the breadth and magnitude of the CD8 T-cell response did not correlate with either control of viral replication or maintenance of normal CD4 T-cell counts.

The session concluded with a presentation that looked at entry of primate retroviruses into humans. This presentation by Nathan Wolfe from The Johns Hopkins Bloomberg School of Public Health began with a discussion of the hypothesis of how the HIV pandemic likely originated by cross-species transmission from SIV infected chimpanzees. It is believed that the emergence of these viruses has been limited by the rarity of successful cross-species transmission. However, little is known about the frequency of retroviral zoonoses and the mechanisms of retroviral emergence. His group examined blood specimens from 930 individuals in Cameroon who were involved in the hunting or butchering of primate bush-meat. Plasma samples from 90 individuals (9.7%) were Western blot reactive and proviral sequences were PCR amplified from peripheral blood lymphocyte DNA of 13 Western blot reactive persons.

Phylogenetic analysis revealed that two primate hunters were infected with two novel viruses (designated as HTLV –3 and HTLV-4). The remaining 11 individuals who had PCR amplified virus were infected with a broad

diversity of HTLV and STLV viruses that had been previously seen in mandrills and other primates, but had not been previously seen in humans. Their conclusion was that bush-meat hunters in Africa who are exposed to infected primates are regularly infected with novel HTVL. They discovered six viruses that had crossed from infected primates, including two novel human retroviral species, HTLV-3 and HTLV-4. They hypothesized that the cross-species transmission following primate exposure is not the rate limiting step in retrovirus emergence and suggested that emergence may be predicted by surveillance of human populations exposed to animal reservoirs.

[Note: For a backgrounder, see http://www.aidsinfonyc.org/fiar/11thCROI-fin.html .]

Cellular and Viral Factors in Virus-Host Interplay

HIVandHepatitis.com (2/28/05) Bailey L

[Report from the *12th Conference on Retroviruses and Opportunistic Infections*]

The oral abstract session "Cellular and Viral Factors in Virus-Host Interplay" looked at a number of novel viral-host interactions that may pose an avenue for future drug discovery and development. There were ten oral abstracts presenting a half dozen different viral-host factor interactions. Three of those factors will be discussed here:

1. APOBEC 3F and 3G
2. TRIM 5a
3. BAF and other nuclear membrane proteins

There were four presentations that examined APOBEC 3F and 3G. Reuben Harris of the University of Minnesota describes APOBEC 3F and 3G as being two of ten APOBEC proteins coded by chromosome 22. There proteins are only found in mammals and the number of different APOBEC proteins increases from only one in the mouse to ten in man. The role of APOBEC 3F and 3G is to limit the activity of retroviral transposons in the cytoplasm of the cell. They accomplish this task by causing a cytidine deamination reaction in the negative DNA strand of the retroviral genome. Deamination changes the cytidine amino acid to uracil. Upon reverse transcription, this leads to a G to A mutation in the retroviral genome. Dating back to the mid-1990s, a number of studies have observed a "G to A hypermutation". In small quantities, these mutations may favor the virus by posing an opportunity for evolutionary escape to certain challenges. But, when seen in large numbers, these hypermutations are highly deleterious to viral survival.

There are multiple pathways by which retroviruses defeat the intervention of APOBEC 3F and 3G. Andrew Mehle of the Dana-Farber Cancer Institute discussed how the Vif protein acts to overcome the anti-viral activity of APOBEC 3G. Vif (viral infectivity factor) does this by assembling a complex of Vif, Cullin 5 protein and E3 ubiquitin ligase. This complex interacts with APOBEC 3G leading to the attachment of multiple ubiquitin molecules to the APOBEC 3G molecule. This ubiquitinization process marks APOBEC 3G for degradation by cellular proteosomes and thus removal from the cytoplasm.

In another presentation, Bärbel Schröfelbauer of The Salk Institute discussed her work with HIV-1 Vpr. She describes this as being yet another viral gene that codes for a protein that has anti-APOBEC 3G activity. She stated that the effect of HIV-1Vpr was independent of HIV-1 Vif activity. She describes two uracil DNA glycosylases (UDG), SMUG1 and UNG2 that have anti-viral activity. The role of these enzymes is to target the hypermutated viral DNA strands that have been acted on by APOBEC 3G deamination (cytidine to uracil) and mark them for degradation. The viral protein coded by Vpr acts by blocking these UDGs from encapsulation in virions. These UDGs are also removed from the cell cytoplasm by the process of polyubiquitinization and subsequent degradation via cellular proteosomes. Ms. Schröfelbauer concluded, "The presence of two accessory genes (Vif and Vpr) in the viral genome dedicated to blocking the action of APOBEC 3F and 3G highlights the importance of cytidine deamination in the viral life cycle."

TRIM 5a was another host anti-viral factor discussed in a presentation by Matthew Stremlau, also of the Dana-Carter Cancer Institute. In his presentation, he discussed the observation that HIV-1 can enter the cells of all Old World primates, but the epidemic is confined to humans and apes. Recently a genetic screen identified the cytoplasmic component TRIM 5a as a major post-entry restriction factor in Old World monkey cells.Rhesus monkey TRIM 5a more potently blocks HIV-1 infection than human TRIM 5a. Analysis of species specific variation in TRIM 5a has identified three variable regions (v1, v2 and v3) within the SPRY domain. Work by Dr.

Stremlau has identified the variation in the v1 region as being responsible for the effective blocking of HIV-1 infection in rhesus monkeys. His work showed that replacement of only three amino acids at the N-terminus of the human TRIM 5a v1 region resulted in a protein that restricted HIV-1 nearly as efficiently as the rhesus type TRIM 5a.

In a final presentation, Jean-Marc Jacque of the University of Massachusetts Medical School discussed the observation that lentiviruses (e.g. HIV-1), unlike onco-retroviruses, are not able to infect actively dividing cells. He observed that when cells are arrested in mitosis, the ability of HIV-1 to integrate into the host genome is markedly impaired. His work showed that this block exists after HIV-1 accesses the nucleus, but before integration, suggesting that a component of the nuclear membrane itself might be required to complete the integration process. He found that BAF and a number of other proteins in the nuclear envelope interact with HIV's pre-integration complexes and the host's DNA to facilitate integration. When these nuclear proteins are silenced by RNA interference, the viral cDNA retains the ability to localize to the nucleus but is unable to integrate and thus unable to replicate.