Notes from the Gallo Lab Meeting

by Mark Harrington
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Late every August, the once-and-future greats (and some not-so greats) of AIDS research pay pilgrimage to HIV co-discoverer Robert C. Gallo at the annual laboratory meeting once, in palmier days, hosted in Bethesda by the NCI Laboratory of Tumor Cell Biology, now hosted by the Institute for Human Virology (IHV) and held at the tourist-friendly Hyatt Regency Harborside in glamorous, sweltering Baltimore, Maryland. Your faithful correspondent attended the first four days of the week- long research gab-fest. Some highlights:

Update on the Pan-American Pandemic.

Fernando Zacarias of the Pan American Health Organization (PAHO), a unit of the World Health Organization, delivered an update on the epidemic in the Caribbean and Latin America . PAHO estimates that 1.3 million people are infected in Latin America, and 300,000 in the Caribbean. The epidemic affects different populations in different countries.

Transmission category	Brazil	Mexico	Caribbean
Men who have sex with men (MSM)	40%	55%	13%
Heterosexual transmission	26%	34%	78%
Drug use/transfusion	26%	9%	<1% (4% in Dom. Rep.)
Other	8%	2%	9%

Mexico had a particular problem with blood transfusion-associated HIV transmission from private, for-profit blood banks, as a result of which the government abolished paid blood donations. The epidemic is growing more slowly in Latin America than elsewhere, but there is a variety of patterns:

Type of local HIV epidemic	Example
Low transmission epidemics	MSM in Peru
Concentrated epidemics	IDUs in Brazil, Costa Rica, Panama, Mexico, Venezuela
Generalized epidemics	Heterosexuals in Haiti, Anglophone Caribbean, Honduras

PAHO has utilized a number of strategies to contain the epidemic since the early 1980s. From 1983- 88 it focused on infrastructure development, working with countries to develop national AIDS programs. From 1989-94 it focused on program consolidation and from 1995 on "expanded" responses. Over time, the 19 Latin American countries have had 117 national AIDS program directors, revealing a rapid turnaround time with an average tenure of just 16-18 months.

Zacarias commented that "I was truly surprised by the amount of men having sex with men. Married men didn't think of it as gay sex, but it led to as much as 17-20% of cases." He claimed that PAHO has changed the social perception of condom use and that by 1995 all countries had access to HIV antibody testing kits (I guess this is what they meant by "expanded""programs). He presented some interesting, though dated, figures estimating the global cost of HIV prevention in developing countries -- said to total $2.5 billion (this was in 1990) -- of which two thirds would go to condom marketing and STD management.

Access to HAART, stated Zacarias, is a "political hot potato. Everyone wants it; there's no money to do it." He also mentioned the roles of machismo, homphobia and the role of the church in obstructing rational AIDS prevention and treatment policies.

Estimated Cost of Antiretroviral Therapy to Developing Countries (assuming no price reductions)		
Regimen	**% of HIV population treated**	**Cost (billions)**
AZT	50%	2.5 - 3.3
	100%	5.0 - 6.6
Triple therapy	50%	6.2 - 9.7
	100%	12.3 - 19.4

Zacarias stated that AIDS cases may be going down in Brazil. The government there is spending $700,000 to provide antiretroviral treatment, including protease inhibitors, to 50-60,000 people with HIV there. He discussed a gradient of HIV clinical management, dependent on a country's given health care infrastructure, the quality of usage, etc. It would begin with HIV counseling and testing, ancillary care (diarrhea, pain management, aspirin, etc.), ramp up to opportunistic infection treatment (especially for tuberculosis), sexually transmitted disease treatment, and prevention of vertical transmission, and finally culminate in comprehensive antiretroviral therapy. Obviously different countries have different resources.

Is Subtype HIV-1C More Virulent? Max Essex of Harvard presented an unconvincing paper attempting to demonstrate that HIV-1 subtype C, which is increasingly prevalent in parts of Africa, is more virulent than other subtypes and more easily transmitted heterosexually. He claimed that some C subtypes have additional long terminal repeat (LTR) and NF-kB viral transcription promotor regions than other strains. This would, he claimed, enable subtype C to grow faster than other strains. However, "there are different Cs; all Cs are not alike."

Laurie Garrett, behaving just like Murphy Brown, approached the microphone and decimated Essex's paper with a historical/epidemiological explanation for the greater preponderance of subtype C in the new South African pandemic. During apartheid, freedom fighters were concentrated in Lusaka and Harare (the capitals of Zambia and Zimbabwe). They were treated like rock stars. There was plenty of sex. In addition, laborers from those countries worked at mines in Namibia and South Africa. Patterns of guerrilla travel, social disruption and migrant labor led to HIV transmission along supply routes, leading to a dramatic increase in HIV in southern Africa. The fact that it was subtype C was just a matter of chance.

Essex nodded wearily, unable to contend with social or historical explanations which undermined his neat laboratory findings.

Pathogenesis of Infection with a Virulent SIV Strain.
Ashley Haase presented a study of early events observed following mucosal infection of rhesus macaques with the virulent SIVmac251 strain. Female macaques were inoculated intravaginally with 1,000 SIVmac251 particles. Local biopsies were taken at days one, three, seven and 12, and assays performed for cytotoxic T lymphcytes (CTLs), viral genotype and virus/host interactions. The animals were soon euthanized to detect spread of SIV throughout the body. By day 3, SIV was detected in mononuclear and dendritic cells in the cervical region. Infected CD4 cells were also found in the intraepithelial region aand in the adjacent lamina propria. By then, quite a few cells had SIV RNA. There was an explosive, 70-fold increase in infected cells during the first week, and another 8-fold increase over the following 12 days, mostly in CD3+ T lymphocytes.

SIVmac251 Cellular Host Range During Early Mucosal Infection			
	Day 3	Day 7	Day 12
CD3+T cells	90%	79%	80%
CD4+T cells	NT	86%	89%
CD45RO+memory cells	NT	80%	84%
CD60+	NT	11%	12%

SIV dissemination is detectable within the first week after mucosal inoculation, with an explosive increase mainly in the lymph node paracortical (T cell) region. The cervix is infected by days 3-7. Everything but the central nervous system is infected by day 12 (lymph nodes, thymus, gut-associated lymphoid tissue).

CD4 Cell Census by Lymphoid Tissue Sampling.

Moving on to recently infected humans (two within two weeks, two within three weeks, seven within less than 90 days, 13 within less than twelve months), Haase and colleagues conducted lymph node biopsies and found that over 90% of the infected cells were CD4-positive. Thus, CD4+ lymphocytes are the predominant infected cell from the very beginning of the infection.

Number of Productively Infected CD4+ T Cells at Various Stages of Infection	
Acute primary infection	90,000,000
Early (<3 months) infection	40,000,000
Asymptomatic infection	23,000,000
AIDS	130,000,000

Overall, by Haase's calculations, 2.5 billion cells are infected, the average virion count per infected cell is 175, and the daily CD4 cell turnover is 80 million per day. During late-stage disease, there are over 10 billion virions produced per day. Most of the body's virus at any given time is archival virus stored on follicular dendritic cells (FDCs) in lymph node germinal (B cell) centers. About 100 billion virions are stored on FDCs at any given time. (They are rapidly cleared after initiation of HAART.)

CD4 Cell Population Dynamics in Uninfected & Asymptomatic Individuals			
	Uninfected	Asymptomatic	Difference (+/-)
Peripheral blood	5 billion | 2%	1 billion | 1%	50% decrease
Lymphoid tissue	200 billion | 98%	100 billion | 99%	50% increase
CD45RA+ (naive)	45%	24%	40% decrease
Ki+ (dividing)	0.4%	1.2%	200% increase
Apoptotic	0.2%	0.4%	100% increase
(Residuum -- undergoing removal after apoptosis)	0.2%	0.8%	300% increase

Lymphoid tissue represents about 1% of total body weight, or 700 grams in a 70 kilogram person.

During HIV infection there is a relative redistribution of CD4 lymphocytes from the peripheral blood and into lymphoid tissue. There are also perturbations in the types and activity profiles of these lymphocytes, including decreases in the proportion of naive CD45RA+ CD4 cells, and significant increases in proliferating and apoptotic cells.

After initiation of HAART, there is a rapid redistribution of cells from lymphoid tissue into peripheral blood and an increase in the number of memory CD4 cells within three weeks. "The initial CD4 changes mainly reflect redistribution." This is followed by a slow, steady, linear increase in naive cells. According to Haase, two thirds of the cell increase is among the naive subset. Meanwhile, cellular proliferation subsides towards normal values. "Whatever damages the source of new cells [during HIV infection], it does not appear to be permanent," concluded Haase.

CD4 Census in HIV-Negative, Positive Untreated and Treated Individuals			
	HIV-Negative	HIV+ Untreated	HIV+ after Six Months of HAART
Excess capacity	7 x 107/day	--	--
Total CD4 cells	2 x 1011	9.7 x 1010	1.2 x 1011
Naive CD45RA+	9 x 1010	2.3 x 1010	3.8 x 1010
Memory CD45RO+	1.1 x 1011	7.4 x 1010	8.2 x 1010
Proliferating*	8 x 108	1.2 x 109	5 x 108
Apoptotic*	4 x 108	4 x 108	5 x 108
Removal**	--	--	--
Turnover of productively infected cells	--	8 x 107	--

* Italics denote the compartment of homeostasis -- proliferation, apoptosis and removal.
** Removal is difficult to measure, as macrophages rapidly engulf apoptotic cells.

Thus, the system is running close to capacity before HIV infection, so the uncompensated loss of productively infected cells at a rate of about twenty million CD4 cells per day results in progressive, although gradual, immune depletion.

Jan Orenstein questioned Haase's technique and results. Is the 700 grams of lymphoid tissue the only source of HIV in the body? Do silver grains on Haase's light-field slides indicate virions?

Haase replied that they calibrated his assay using cells infected with a known quantity of virus, finding that one grain denoted one virus.

Orenstein then noted that follicular dendritic cells don't trap viral RNA in a vacuum, they trap antigen/antibody/complement complexes and RNA. He asserted that RNA disappears first, but antigen persists. Haase replied that FDCs do trap virus particles.

Treatment of Acute Primary Infection Preserves HIV-Specific CD4 Cell Responses.
Eric Rosenberg gave an update on the work of Bruce Walker's lab at Massachusetts General Hospital (MGH) on preserving HIV-specific CD4 cell activity by treating individuals during acute primary infection (API). They started from the murine LCMV (lymphocytic choriomeningitis virus) model, in which cytotoxic T lymphocyte (CTL) activity is inversely proportional to viremia: high CTL count means low viremia, and vice versa. This also appears to be the case in HIV infection.

However, CTLs are dependent, ultimately, on CD4 T cell help in order to function optimally. Unfortunately, HIV-specific CD4 cells appear to be deleted within the very early months of infection. So far, they do not appear to return even after months of HAART, despite the return of CD4 cells specific to other antigens.

Walker, Rosenberg and colleagues then looked to see whether HIV-infected long-term non-progressors had strong CTL responses to HIV. The answer was yes. He showed CTL data from a rapid (four year) progressor, who had a very narrow CTL response, versus a non-progressor infected for twenty years whose viral load remains below 400 copies/ml who has a very broad CTL response. The rapid progressor demonstrated a lymphocyte proliferative response (LPR) to tetanus but not to HIV, while the non-progressor's cells proliferated in response to p24, gp160 and tetanus.

Next they looked at the relationship between plasma viral load and LPR to p24. These were inversely correlated in two cohorts from Boston and San Francisco.
Their next hypothesis was that an HIV-specific CD4 T helper cell response is generated during acute primary infection (API) and then lost. They took someone with API and treated him with HAART. At baseline, he had no LPR to p24, wherease at three months he did. This LPR developed as the plasma viral load dropped.

Now they have a cohort of twenty individuals followed from very early after infection. Most were antibody negative with plasma viral load over one million at baseline. After initiating HAART, most developed a viral load beneath the limit of quantitation by eight weeks. Eighteen of twenty (90%) had no adverse events. Two developed alopecia; their hair grew back, however.

p24 Lymphoproliferative Response (LPR) in HAART-Treated API Patients		
Time point	**N (%) with p24 LPR**	**Stimulation index (SI)**
Baseline	4/20 (20%)	Not given
2-3 months	11/20 (55%)	28
6 months	10/10 (100%)	36
12 months	4/4 (100%)	30-167

The response appears to be durable. They mapped the target epitopes. The long-term non-progressor and those treated during API recognized similar p24 epitopes. The LPR response to viral envelope proteins was more variable.

Rosenberg, Walker and colleagues also investigated five patients from Franco Lori's Berlin cohort treated with ddI, hydroxyurea and a protease inhibitor. Among those untreated, just one in four (25%) developed a p24 LPR at six months (he was "exceptional"), versus 4/4 (100%) on the ddI/HU/PI combination. One by now notorious individual developed "an appropriate immune response to control the virus in the absence of treatment." Nineteen months after stopping HAART his viral load remains beneath the limit of quantitation. He has both a p24 and an gp160 LPR.

They are now using the new tetrameric epitope binding CTL assay to examine which epitopes are recognized among individuals with HLA-A2.1. By ELISPOT, up to 2,500 of one million PBMCs were interferon gamma-secreting epitope-specific CTLs. However, Daar and Giorgi reported a second episode of acute primary infection (API) when a patient whose viral load became undetectable after six months on HAART stopped therapy. This rebound, including symptoms of API, was associated with a CD8 T cell-mediated immune response (Ann. Intern. Med., 5.15.98).

Rosenberg closed by questioning whether we need to eradicate every infected cell, or whether

immune control can be restored.

Someone asked what happens to the CD4 LPR during chronic infection. Rosenberg replied that it came back in exceptional cases.

Someone asked if there is something special about p24. If you looked at other HIV proteins, what would you see? Rosenberg answered that they have looked at nef and RT LPR and haven't seen anything. They haven't been able to correlate gp160 LPR with plasma viremia either.

Half-Life of the Third Compartment.
Robert Siliciano, discoverer of the so-called "third compartment" of resting, latently infected CD4 cells (L cells), presented new data showing that the third compartment is established at stable levels very early in infection, prior to seroconversion. He presented further follow-up data on his cohort of 22 HAART responders treated at Johns Hopkins. By now they have beween 0.1 and ten latently infected, resting CD4 cells (L cells) per million cells. Sometimes virus can't be isolated from these patients.

The assay has a wide dynamic range; its limit of detection is determined by the number of cells which can be extracted from the body. This is becoming the major problem limiting efforts to quantify very small numbers of reservoir cells. If there are 105 (100,000) L cells, we need a five log reduction to eliminate this population, yet we are already at the limit of detection for L cells. In addition, the assay varies by about +0.7 log, so it isn't too precise.

If the reservoir of L cells contains 105 cells, with a half-life of 8.8 months, the time-to-eradication (TTE) would be on the order of twelve years. "After all, these cells are designed to persist." This assumes that no new L cells are infected during this time. Siliciano hoped that people with acute primary infection (API) would have fewer L cells, but, while there appears to be a trend in this direction, it is not a major difference. The reservoir is filled very early. Over time, it becomes a library of previously circulating viral strains.

Half-Life of Various HIV-Infected Compartments	
Free virus	6 hours | 0.25 day
Actively infected CD4 cells	24 hours | 1 day
CD4 cells with unintegrated virus	6 days
Macrophages	14 days
FDC-trapped virus	14 days
L cells	240 days

Is the frequency of L cells higher in HIV-infected children (who have more CD4 cells)? No.
Siliciano also asked whether R5 (M-tropic) viruses, the predominant strain early during infection, can infect resting naive CD4 cells. The answer, unfortunately, is yes, under appropriate conditions. In vivo, during acute primary infection, large numbers of infected naive cells are detected. In these cells, the virus is not yet integrated into the host cell genome. (If the provirus remains in the cytoplasm, it is degraded by cellular proteases within a week or so.) Fewer than 0.01% of resting CD4 cells harbor HIV provirus; of these, even fewer are replication competent.

Accelerating Decay of the Third Compartment.
Several teams are wasting no time attempting to explore immuno-stimulants which may accelerate decay of the third compartment. At NIH, H. Clifford Lane and Anthony S. Fauci took thirteen patients who had maintained a viral load below the limit of quantification on HAART and administered relatively high-dose interleukin-2 (IL-2). IL-2 is postulated by some to activate resting T cells, although some (e.g., Siliciano) maintain that the resting cell population does not

express IL-2 receptors. In any case, Fauci reported that provirally-infected cells could no longer be isolated from three of the 13 IL-2/HAART recipients, even when as many as 300 million cells were extracted. Obviously the "limit of detection" for eradication experiments will be limited by the impossibility of sampling such numbers of cells. Joep Lange of the University of Amsterdam administered an even more daunting regimen of five antiretrovirals plus IL-2 and the mouse monoclonal antibody OKT3, which targets and activates all the body's T lymphocytes through the CD3 receptor. The result is a clinical condition resembling toxic shock, as all the cells release pro-inflammatory cytokines and many die of apoptosis. The hope was that the coadministered HAART would prevent the viruses produced by waking latently infected cells from infecting another generation of cells. While the patients became very sick (requiring admission to the intensive care unit), infectious virus could no longer be cultured from two of the three. One declared himself cured and disappeared from follow- up. However, in both Lange's and Lane's experiments no one has yet to go off HAART. Lane will start enrolling a study at NIH this fall in which anyone who has maintained a CD4 count below 5 copies/mL for over six months will have their antiretroviral therapy terminated. Researchers will then follow the brave volunteers for evidence of viral re-emergence (this approach does not seem to make sense unless the patients have received immunostimulatory therapy). Researchers were reluctant to use the "C-word" in their presentations, but the halls were abuzz with the slightly surreal gossip about very toxic, potentially fatal, possibly curative regimens.

New Evidence for Thymic Reactivation in Adults on HAART.

Richard Koup of the University of Texas (formerly at Aaron Diamond) developed a new technique for measuring how recently new T cells emigrated from the thymus.

Circular fragments of DNA which are produced in T cells during thymic maturation, known as alpha circles or T cell rearrangement excision circles (TREC), can be used to measure how recently a T cell emerged from the thymus. Recent thymic emigrants have high levels of TREC, which are formed by excision of DNA fragments during the forming of the T cell receptor during T cell receptor maturation. The TREC DNA fragment copy number is diluted by half with each round of cell division. Therefore, quantitating TREC appears to be a surrogate marker for recent thymic emigration (RTE). People who have had thymectomies have decreases in their amount of TREC, suggesting that thymic activity persists into adulthood.

Koup applied the new assay to the T cells of people receiving HAART and found that as viral load decreased to beneath the limit of quantitation and CD4 counts rise, the amount of TREC in the new CD4 cell population rises as well. This rise is seen exclusively in naive (CD45RA+) T cells, suggesting that they are indeed maturing through the thymic pathway. The TREC increase seen after HAART could only occur if 1) the thymus is active in adults or if 2) lymphocyte receptor maturation happens extrathymically. Koup is now collaborating with Barton Haynes at Duke University to apply the TREC approach to studies of thymic function in HIV infection and other immunologic conditions.

Macrophage Infection May Be Essential for Retroviral Pathogenesis.

Mario Stevenson of the University of Massachusetts Medical Center at Worcester presented a fascinating lecture on several aspects of retroviral infection of monocyte-derived macrophages in HIV and SIV infections. He stated that the antigen presenting celll (APC) is "the second class citizen" of AIDS research, but may be essential for the infectivity of primate lentiviruses. He suggested that these primate retroviruses may change the morphology of the APC to ensure spread of new virions. He also pointed out that in patients with AIDS dementia complex (ADC), the predominant infected cell is the microglial cell, which is a brain macrophage.

Thus, lentiviruses have a long term relationship with long-lived cells of the monocyte lineage. If a cell is not undergoing mitosis, how can HIV integrate its DNA into cellular DNA by passing through the nuclear membrane (which, conveniently is dissolved during cell division)? Yet we know that HIV infects non-dividing macrophages. How is this accomplished? Several viral proteins facilitate transclotion through the nuclear membrane. The HIV structural matrix protein

(MA, matrix antigen) facilitates nuclear translocation; a region of this protein binds tightly to viral RNA. MA activity is additive with that of vpr, another viral protein with nucleophilic properties.

Stevenson and colleagues set out to try and construct a virus which could not productively infect macrophages. They were able to do so using an SIVSMM related to HIV-2. Elimination of the analogous protein vpx in the SIVPBJ strain was able to eliminate the ability of SIV to infect monkey macrophages.

Vpr delays G2 cell cycle arrest. It is also associated with a DNA repair enzyme. In SIVMAC, however, these two activities are segregated in vpr not vpx. Stevenson and colelagues made SIV mutants. Those with vpx deleted (Dvpx) were impaired in their ability to infect macrophages, where those with vpr deleted (Dvpr) experienced impaired cell cycle arrest.

They infected monkeys intravenously or intrarectally with these gene-deleted viruses and measured plasma viral load and lymphocyte number.

Impact of vpr or vpx Deletion in SIVPBJ Infected Macaques			
Route	**Strain**	**N**	**Result**
IV	PBJ WT	1	Dead within ten days.
IR	PBJ WT	1	Dead within 13 days.
IV	Dvpr	2	One died, one survived.
IR	Dvpr	2	One died, one survived.
IV	Dvpx	2	Viral load 2-4 logs lower than WT.*
IR	Dvpx	2	Viral load 2-4 logs lower than WT.*

IV = intravenous; **IR** = intrarectal; **PBJ** = SIVPBJ, a super-pathogenic strain; **WT** = wild-type.
* 100-10,000 copies/ml rather than 10-100 million.

However, because it is competition between viral strains which drives viral evolution, not competition to kill the host, it was necessary to coinfect animals with WT and Dvpr or WT and Dvpx to determine which strain predominated. Equal amounts of each strain were inoculated either intravenously or intrarectally, and the animals were followed. In this case, coinfection did not slow down viral replication. No recombination was observed.

With WT/Dvpx coinfection, at day five most viral clones (thousands) were WT, while only six were Dvpx. Thus, there was rapid selection against Dvpx within the first six days of infection. No Dvpx clones were detected by day seven.

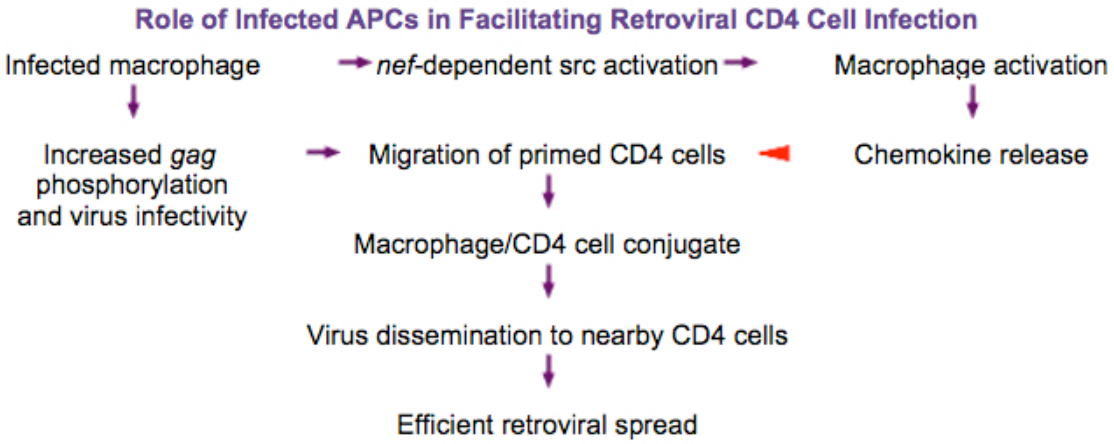
With WT/Dvpr coinfection, there was less selection against the mutant strain. Dvpr was much more common, amounting to 15-25% of viral strains by days 9-12. However, the monkeys died just as quickly as control animals.

Thus, a viral strain impaired in its ability to infect non-dividing cells, such as the SIVPBJDvpx, can infect animals mucosally, but has trouble traveling to distant sites in the body. In contrast, the ability to infect macrophages (and perhaps dendritic cells) promotes the virus' ability to spread to distant sites. (This hypothesis is in line with the proposal of Grossman, Feinberg and Paul in the May PNAS.)

Stevenson speculated that the macrophage or dendritic cell provided a useful substrate for spreading the virus from T cell to T cell: an infected antigen-presenting cell would recruit CD4 cells and viruses produced within the APC could then infect a number of CD4 cells recruited by

proinflammatory chemokines. Perhaps HIV even induces the macrophage to produce chemokines in order to recruit CD4 cells. Infected macrophages release MIP-1a, MIP-1b and M-CSF as they make virions. These chemokines attract CD4 cells. (RANTES is not involved.)

How might HIV (or SIV) induce macrophage chemokine release? One possibility is that the nef protein binds to the Hck macrophage cellular kinase (lymphocytes have a similar Lck kinase). Nef contains highly conserved motifs which interact with the Src/Hck pathway, thus activating myeloid cells. Stevenson showed some elegant lab-work indicating that nef does indeed interact with this pathway. Thus, nef activates macrophages which produce virus and recruit CD4 cells in order to infect them. This greatly facilitates retroviral infection. However, this needs to be shown in vivo.



Frequent HIV Reinfection Demonstrated in Chimpanzees.
Addressing an issue plaguing HIV treatment and prevention, Pat Fultz of the University of Alabama at Birmingham (UAB) presented compelling evidence that infection with a primary HIV strain does not prevent subsequent reinfection by another strain in the chimpanzee model. In some animals, both strains were readily detectable at later time points, while in some cases the earlier strain (or strains, in one animal reinfected twice) predominated. The ability to detect superinfection depends on using strain-specific PCR primers (which is not done with standard commercially-available viral load assays used in humans). Fultz stated that "when both strains are minimally pathogenic, the first strain predominates in multiple tissues at death," reminding us that "minimally pathogenic"in the chimp is equivalent to an average pathogenic strain in humans (e.g., ten years to clinical disease). This work has chilling implications for the dissemination of drug-resistant HIV strains among indivudals already infected. Superinfection is not necessarily detectable by increasing antibody titers, suggesting that there is not always a "booster effect". Fultz achieved 100% (9/9) superinfections within viral clades, and 9/13 between clades.

Powerful New CTL Assay Detects SIV-Specific CTLs in Macaques.
Norm Letvin of Harvard presented an exciting new tetramer assay which detects SIV-specific cytotoxic T lymphocyte production in rhesus macaques during immunization with an attenuated SIV strain and subsequent challenge with a more pathogenic strain. CTL activity detected in blood was proportional to (but greater in magnitude) than CTL activity in lymphoid tissue. CTL activity is detectable within eleven days of infection and decays rapidly as viremia is cleared. This tetramer assay will be essential for monitoring CTL activity in vaccine trials. (Rosenberg and Walker are using it in their treatment trials as well.)