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YOKOHAMA REPORT

HIGHLIGHTS OF THE Xth INTERNATIONAL CONFERENCE ON AIDS/Vth STD CONFERENCE

held August 7-12, 1994, Yokohama, Japan.

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This document is an information resource. Treatment issues relating to HIV are complex and evolving. Individuals should gather as much information as possible about a therapy before making a treatment decision in consultation with a health care practitioner. Persons relying on information contained in this document do so at their own risk.

YOKOHAMA REPORT

This was not a conference that announced any significant breakthroughs or innovative new therapeutic approaches. Rather there were presentations of new information related to disease progression and treatment approaches, like proteinase inhibitors and gene therapy, which have been under study for some years now. Current inadequacies in the treatment of HIV infection remain largely due to the wide gaps in our understanding of the pathogenesis of HIV disease.

LONG-TERM NON-PROGRESSORS (LTNP)

It is now estimated that about 5% of HIV positive people are long-term non-progressors. These are defined by Tony Fauci of the NIH as individuals who are seropositive, with CD4 counts above 500, a slope of change in CD4 counts equal or greater than 0 for more than 7 years, and who have received no antiretroviral treatment.. Lymph node architecture is completely preserved and lymphoid tissue is remarkably well preserved in these individuals. This is in sharp contrast to progressors. With respect to viral burden LTNP's had a consistently lower burden in peripheral blood and nodes (by about a log (10 fold)) than progressors and the level of replication is much lower. This suggests that in the face of a replicating virus, it is the host's response to the virus vis-a-vis virus trapping, tissue reaction to virus and perhaps the initiation of immuno-pathogenic processes in the lymphoid tissue that likely play an important role together with the competency of the virus in the evolution of the state of long term non-progression. LTNP's appear to have a robust immune response to HIV.

Dr. David Ho presented the results of his studies of LTNP's (defined as 12 years HIV positive, with normal and stable CD4 counts and no symptoms). Quantitation of plasma viral RNA showed levels well below those found in patients with disease progression. These individuals exhibit markedly better neutralizing antibody response than do individuals with progressive infection and a very potent cellular immune response is also present. Depletion of specific CD8+ cells from nonprogressor samples resulted in marked growth of virus in nonprogressor cells . Sequential addition of each subject's own CD8 cells to the CD8 depleted cultures resulted in dramatic return of suppressive effect. CD8 cells are the key to the effective immune response of LTNP's.

DISEASE PROGRESSION

AIDS was originally thought of a monotropic (caused by one virus) disease. It is now thought to be polytropic (caused by various strains of one virus), multifactoral (many co-factors influence progression), and multiphasic (there are many phases to progression). A number of co-factors are thought to play a role in disease progression including the genetics of the host, the host's immune response, cellular activities, cytokine secretions and immunocytotoxicity. It is cytokine signals which may lead to apoptosis (cell death) and anergy (inability to react to antigen presentation). Production of IL-6 and TNF-alpha are heightened at all stages of disease while IL-2 is at low levels. There is hyperactivity and expression of pro-inflammatory cytokines at all stages of HIV disease. There is no obvious shift from early to late disease, leading Fauci to question the theory that there is a shift from TH1 to TH2 part way through HIV infection.

The major receptors for HIV are CD4+ cells (as well as Gal/c, Fc and complement receptors). CD8 + cell antiviral factor (CAF) blocks viral transcription and a high level of CD8+ cells correlates with a good clinical state. The CD8+ cell antiviral response is enhanced by TH1 cytokines, such as IL-2 and inhibited by TH2 cytokines such as IL-10. Reduction in cytokines caused by TH2 can be reversed by TH1. A decrease in IL-2 which is important in the growth and function of CD8+cells and an increase in the production of IL-4 and IL-10 seem to lead to progression of disease.

Dr. Haasse presented evidence that there are staggeringly large numbers of CD4 lymphocytes and macrophages in the lymphoid tissues (eg. lymph nodes, tonsils, gut-associated nodes) that harbour the HIV provirus in a silent state that allows infected cells to go undetected by immune surveillance. 25% of CD4+ cells in pre- and early symptomatic disease harbour HIV DNA but only !% or less of CD4+ cells have copies of RNA as virus-producing infected cells. This means that there are about 100 billion CD4+ cells covertly infected and 1 billion productively infected. A war of attrition occurs with input of new CD4+ cells from the thymus at half the rate that cells are killed.

He outlined HIV disease progression as follows: the virus enters the body and circulates in the blood, productively infecting macrophages; it also circulates through the lymphatic system and acts as a cell-associated virus, establishing itself in CD4+ lymphocytes and allowing infected cells to escape immune response. The viral burden at this stage is incredibly large. Activation of viral gene

expression in vivo (in the body) is an ongoing process that results in a very large pool of productively infected cells that spreads infection and contributes to the slow depletion of CD4+ lymphocytes. Dr. Gallo said that the realization that there was an incredibly large number of latently infected cells constituted a powerfully new change in perception which makes direct antiretroviral therapy a must.

There are different phenotypes of HIV. Some replicate slowly (called slow viruses), others rapidly. Some don't kill cells (low cytotoxicity) while others develop syncytia (large clusters of infected cells) and kill other cells in culture. Slow/low virus is maintained in about 50% of AIDS patients, while in the other 50% it changes to rapid/high virus. Individuals with rapid/high virus have significantly lower CD4 counts and progress more quickly to disease and do not benefit as much from AZT therapy.

GENE THERAPY

Gene therapy remains the most promising approach for a cure to AIDS. The basis of gene therapy derives from the fact that in HIV infection the virus makes use of the host cell genes to replicate itself. The model of gene treatment for HIV is as follows:

- create in the laboratory a gene that is defective for HIV replication but does not affect host cell function;
- attach the gene to viral DNA that does not replicate itself in humans;
- infect (transduce) donor lymphocytes and macrophages or stem cells;
- infuse these resistant cells into HIV-infected patients.

In the ideal case the newly infused cells would gradually replace HIV infected cells and prolong the healthy state. HIV would eventually run out of susceptible host cells to infect.

Dr. Flossie Wong-Stahl received strong approval last year in Berlin for her statement that it was time to move from the laboratory bench to the bedside. Unfortunately little progress has been made in her clinical studies which she attributed largely to regulatory hurdles.

Dr. Wong-Stahl has created a ribozyme gene with an RNA cleaving property that gives it an antiviral effect. This gene was inserted in a murine (derived from a mouse) leukemia virus previously used in gene therapy. Ribozyme is used at the entry of viral RNA and at the time of packaging, thus making this a combinatorial approach. Her group is refining gene constructs and

delivery vectors and simultaneously proceeding with a six person Phase I clinical trial.

There are at least nine US biotech companies currently developing gene therapy products for AIDS. A group at the University of Toronto has also developed a product which it hopes to bring to Phase I study in about six months time.

GEM 91 is an antisense (antisense blocks out existing genetic instructions) compound which binds to the conserved region of HIV. Phase I studies in the US and France have shown it to be safe. It is expected that its first efficacy data will become available in about 6 months time.

ANTIRETROVIRAL THERAPY---EARLY COMBINATION THERAPY??

Several speakers drew analogies to anti-tuberculosis treatment with streptomycin as a monotherapy in the 1950's. There was a 35% relapse rate which was eliminated when this drug was used in combination with other TB drugs. For HIV the present regimens with one drug alone or one class of drugs cannot be successful in the long run because of the development of resistance. Accordingly Dr. Stephano Vello of Italy predicted that in the near future we will likely see the rapid development of combination therapy with 2 or 3 antivirals working through distinctly separate mechanisms. Whether combinations will result in a delay of resistance is not yet demonstrated in HIV. And some studies in advanced AIDS cases pre-treated with monotherapies have found combinations not to be beneficial. Moreover combinations may have to be carefully chosen; in one study 1 of 4 patients who received alternating weekly regimens of AZT and ddC developed resistance to both drugs. The interactions among various resistance-conferring mutation sites are complex and will have to be worked out carefully in in-vitro tests, at the same time as clinical studies proceed.

Dr. Vella also predicted that treatment would begin earlier in HIV infection, that there would be more individualization of therapy based on laboratory markers and that supplemental immunotherapy would become commonplace. In support of early intervention one American study of patients with primary HIV infection (the acute illness shortly after initial infection with HIV) found that all parameters of CD4, CD8, and p24 favoured the AZT group over a placebo group and that 7 of 8 clinical illnesses were in the placebo group. Virus was detected in all samples at any time however. Vella ended his talk by invoking the model of curative treatment for acute leukemia. Such a model applied to HIV infection postulates initial

high-dose combination antiretroviral drug therapy as early as possible after initial infection in an attempt to reduce viral load to levels that, with follow-up lower-dose maintenance drug therapy combined with immunotherapy, might possibly be curative.

ANTIVIRAL THERAPY---PROTEINASE/PROTEASE INHIBITORS

In the first half of 1995 compassionate access will likely be provided to two proteinase (or protease) inhibitors. The initial antiretroviral drugs were all inhibitors of reverse transcriptase, the HIV enzyme that is the key to the virus' entry into the nucleus. The proteinase inhibitors are a newer class of drugs whose locus of action is at the point where the virus is replicating and being released from the host cell.

Several dozen compounds have now been made by almost 20 pharmaceutical labs. They vary considerably in their anti-HIV activity and bioavailability. Some have a short half-life and do not cross the blood-brain barrier. Also resistance has been demonstrated which likely means combination therapy will be necessary.

The Roche proteinase, **saquinavir** (formerly Ro 31-8959), is the best known inhibitor and has now been studied in 400 patients in 4 phase I/II studies. The recently completed ACTG 229 study found that the triple combination of **saquinavir, AZT and ddC** was superior in terms of immunologic and virologic markers than either of the two double combination regimens or monotherapy. There was no difference in toxicities. **Saquinavir** alone produces a decrease in viral load comparable to AZT. A Phase III study (with **ddC**) is currently enrolling at 4 Canadian sites and another Phase III study (with AZT) is planned to begin in autumn. A compassionate arm is expected to open in the first half of 1995 but supply will be limited and the drug will be rationed. The Roche representative also acknowledged that they are working on a new formulation of **Saquinavir** that will have a better bioavailability, which will be the product that they hope to bring to market.

The Merck protease has good oral bioavailability and has a more dramatic impact on viral load than the Roche product. Results from a Phase II study should be available by the end of the year comparing different doses of the protease to combinations with AZT and **ddI.**

Several speakers emphasized the need to study proteinase inhibitors in combination. The Roche representative acknowledged that the Roche and Merck products would work well together but this would have to be a decision of the InterCompany Collaborative

Group. There is a real fear in the community that people will be using these drugs in combination long before the companies get their act together.

ANTIVIRAL THERAPY---REVERSE TRANSCRIPTASE INHIBITORS

Dr. Volberding summarized the current status of antiretroviral therapy. Clinical use of AZT monotherapy is not recommended for asymptomatic HIV adults with CD4 counts greater than 500, although the rationale for therapy in this group remains very strong. The resolution of the debate when to initiate therapy awaits the development of more effective and durable treatment agents.

Two studies showed some value in switching to **ddI** after early AZT monotherapy in terms of clinical benefit which lasted over one year.

In a study of **d4T** in advanced AIDS patients one half experienced some type of adverse event and less than a third experienced any CD4 increase.

Another study showed that **acyclovir** taken consistently at a dose sufficient to suppress herpetic recurrences (600-800 mg/day) can have a significant impact on prolonging survival.

A report was given on **delavirdine** (U90) from Upjohn which, used in combination with AZT or **ddI** or both, is well-tolerated and has anti-HIV activity. 30% of participants developed a skin rash which was dosed through in 85% of cases. A Phase III study is expected to proceed in Canada this fall.

Other agents under investigation include hydroxyurea, L-FDDC, SID 791, sulfated colominic acids, synthetic peptides, prednisolone, AG1343, ALX40-4C, GASP-1 and loviride. Some very early work is being conducted on integrase enzyme inhibitors as well.

MOTHER-TO-CHILD TRANSMISSION

Several factors influence the risk of transmission: maternal age, nutritional status and coinfections, conditions of delivery and the presence or absence of certain HIV antibodies. It is well established that CD4 counts correlate with risk of transmission: less than 200 gives a 45% risk, over 500, a 15% risk. A study was presented suggesting that 35% of HIV positive babies were infected before birth while 65% were infected during birth. Also first-born twins are at greater risk than their later-arriving siblings, suggesting that increased time in the vaginal canal may result in greater infectious exposure. Delivering babies with attention to HIV disinfection of the

birth canal and by cesarean section should be considered. A large international study is to begin to determine whether CS reduces transmission rates significantly.

Results of ACTG 076 which showed that AZT reduced perinatal transmission were presented. At 18 months the transmission rate was 25% in placebo cases and 8% in AZT cases. Community representatives voiced some concerns about the use of this therapy.(long term effects of AZT on infants, drug resistance, timing of transmission, same efficacy without also treating infants, applicability of findings to countries with lower transmission rates etc.) It may be that combinations more effective than AZT alone would be better and active and passive immunization needs to be investigated.

OPPORTUNISTIC INFECTIONS AND MALIGNANCIES

Kaposi's sarcoma • Robert Gallo said that KS spindle cells seem to be hyperplastic endothelial cells activated by inflammatory cytokines, especially TNF-alpha and IL-6. Spindle cells produce basic fibroblast growth factor which induces angiogenesis which appears in KS. Early pregnancy sera from humans blocks KS-Y1 cell growth. Human chorionic gonadotropin and subunit beta-HCG also seem to block KS spindle cell growth with exquisite specificity.

•A Phase II study of **Daunoxone** found that 14 of 42 achieved a partial response while 28 of 42 remained with stable disease.

•One study recommends using IL-4 in the treatment of KS.

Candidiasis • **Itraconazole** was found to be effective in 63% of **fluconazole** refactory cases of oropharnygeal candidiasis. Cross-resistance appeared to account for failed therapy in 50% of cases.

CMV • Intravitreal ISIS 2922, an antisense compound, which acts on messenger RNA was found to be safe in a Phase I/II study. It is effective against virus resistant to **ganciclovir** and **foscarnet**. Out of 16 patients, 10 experienced ocular inflammation and 8 experienced blurred vision. Phase II/III studies are proceeding. No compassionate access is available.

•prophylaxis is important for people with counts below 100 with one study showing a 2 year incidence of CMV of 32%. **Oral ganciclovir** is being studied for this purpose. There is no compassionate access at this point in time.

AIDS dementia complex • **ateviridine** was shown in one study to be effective in treating ADC. In 4 of the 5 patients who completed the protocol the mean combined impairment score dropped from 19.6 to 5 over a twelve week period.

Diarrhea • A comprehensive evaluation of patients with chronic diarrhea identified pathogens in the gastrointestinal system in 55% of cases. Cryptosporidiosis and microsporidiosis were the most frequent . An endoscopy was required only to diagnose CMV.

PCP • Another study shows the dramatically decreased incidence of PCP and toxoplasmosis for those who were successfully desensitized to **septra/bactrim.** 84% were successfully desensitized. Of those who failed desensitization 50% developed PCP/toxoplasmosis and 25% died, despite using **aerosolized pentamidine.** The researcher recommended that both dapsone and AP be used in patients who fail desensitization.

Cervical cancer • was described as a multifactoral malignancy. Human Papilloma Virus is found in the majority of cervical lesions but other co-factors seem to activate the malignancy, including hormones, carcinogens and immune suppression. One researcher felt that the time is right to develop a therapeutic vaccine which activates HPV specific t cells and it was predicted that we will have interesting news over the next 5 years or so on this.

VIRAL LOAD AND RESISTANCE TESTS

Molecular techniques of PCR and IHC (immunohistochemistry) allow us to detect CMV, HSV, PCP and HIV in tissue and stools where previous standard techniques failed to detect an infectious agent. Viral burden is highly associated ("highly predictive" according to one researcher) with the development of drug resistance and death in patients on long term therapy. People with less than 1000 copies of RNA per millilitre had little risk of death, while those with more than 10,000 copies per millilitre had a 40% risk of death per year. One study reported that less than 1000 copies per millilitre was associated with no evidence of resistance and greater than 100,000 copies was associated with the appearance of resistance at a rate of 38% per year.

Viral load may also be used to measure the effectiveness of the treatments you are receiving although this has still not been clinically validated. One study reported that HIV RNA had a 90%

predictive value, whereas CD4 count had a 37% predictive value for treatment effect. Another study found that a decrease in viral copy numbers is associated with a 40% decrease in the risk of disease progression.

The use of these tests could revolutionize HIV medicine by improving the treatment people receive and by shortening the time required for new treatments to be proven. When a person is about to start a new antiviral drug or change regimens, it is now possible to get a good idea, within about a month, of how well the new treatment is working for them. The tests might also be useful in measuring the usefulness of immune-based therapies. And the biggest positive result will be the ability to test the hundreds of complementary treatments used by individuals and if they provide a striking effect resources can be mobilized to conduct a formal study.

Two viral load measures became commercially available in the last year. One is Chiron's Quantiplex branched DNA (available since August 15) and the other is Roche's molecular reverse transcriptase RT-PCR(available since December 1993). Both of these tests are highly correlated. The Chiron assay has a niche in late stage disease while the Roche assay has a more dynamic range.

Work in validating RNA assays against clinical endpoints is proceeding slowly. Some work is being done in British Columbia to relate CD4 counts, stage of disease and viral load measures. These tests are not yet in general clinical use in Ontario but it is hoped that they soon will be.

OTHER DIAGNOSTIC TESTS

The failure to accurately diagnose AIDS-related conditions in a timely manner continues to be one of the biggest problems in the treatment of people with AIDS. The asymptomatic presence of life-threatening infections makes early diagnosis essential. One study found that 41% of patients with a positive cryptococcal antigen denied headache and fever symptoms. 42% of patients with a positive MAC stool culture denied chronic diarrhea. Another study found that a high % of PCP positive patients have copathogens. A significant number of pulmonary pathogens are not identified in routine sputum examinations and early bronchoscopy is needed. Lack of response to therapy may not be due to drug failure but to co-pathogens.

One study found a good correlation between the results of pap smears as compared to endoscopy in screening for cervical cancer.

Good results were reported from TEICH TARGET, a self-screening test for CMV retinitis.

WASTING AND NUTRITION

Sorono presented the findings of its study of human growth hormone. Those in the growth hormone group gained an average of more than three and a half pounds over three months. The average gain in lean body mass was more than six pounds. There is a risk that the hormone will also start malignancies, like Kaposi's Sarcoma, growing. Another Phase III study is currently underway with 180 patients to garner further evidence of safety and efficacy before this product receives marketing approval.

Thalidomide, at the dose used in one study, had no in vivo effect on HIV viral burden but did significantly reverse the natural history of wasting syndrome.

As usual, studies on nutrition (and complementary therapies) took a back seat to pharmaceutical strategies at this conference. A number of abstracts did confirm a range of nutritional deficiencies in anti-oxidants such as glutathione, and micro-nutrients such as selenium, zinc, magnesium and B vitamins. Supplementation with NAC (for glutathione) was reported to increase t cell levels and to decrease apoptosis (early cell death). Magnesium supplementation benefited patients suffering peripheral neuropathy. A John Hopkins study found taking up to five times the recommended daily dose of B vitamins was associated with decreased mortality and increased survival in a group of PWA's.

COMPLEMENTARY THERAPIES

Although there were a number of small unconfirmed studies showing benefits for different herbal mixtures, several studies provided evidence to indicate the utility of three available complementary therapies: Glyke, Glycerrhizin (both are licorice based Chinese herbs available in concentrated form in Japan) and DNCB (a photo chemical that stimulates certain kinds of cell mediated immune response). Further study is clearly needed.

INFLUENZA VACCINATIONS

Current guidelines call for the vaccination of people with HIV with influenza and pneumococcal antigens. Vaccination appears to lead to activation of t cells and a 14 times increase in HIV virions,

which then decreases. Further investigation is needed to determine whether these guidelines should be reconsidered.

VACCINES

No really promising vaccine against HIV is on the horizon. There are some however who believe that there are vaccines and what is lacking is the will to try them out. They are angry at America's recent decision not to proceed with a Phase III study of the gp120 Genetech vaccine and hope that other countries will be bolder.

CYTOKINES

Cytokines are substances (peptide mediators) released by macrophages, monocytes and lymphocytes which affect the function of other cells. They act as up- and down-regulators of the immune system, modulating the growth, mobility and differentiation of white blood cells and other cells. Several cytokine and anti-cytokine therapies are being investigated, including IL-2, IL-12 and the TNF-inhibitors, and pentoxyfylline. Clinical investigators are also expanding HIV-specific cytotoxic t-lymphocytes for re-infusion into the infected donor of these cells.

SUBTYPE O OF HIV-1

A new subtype that does not fit any of the older subtypes, A to H, has been identified and labeled O for "outlier" to emphasize the distance of each of these variants from known HIV-1 subtypes. This subtype reacts only weakly to the presently constituted HIV1/2 antibody test resulting in a significant incidence of false negative tests. At present the subtype is very rare outside Cameroon where it presently constitutes 1% of all new HIV cases.

FUTURE RESEARCH DIRECTIONS

Robert Gallo thought that four approaches need to be explored to avoid the problem of escape mutation. First, antisense seems to depress escape mutation formation. Second, gene therapy employing inhibitory molecules targeting CD4+ T cells and ultimately the stem cells should be pursued. Third, virus versus virus therapy employing human herpes virus7 which may block CD4 receptors seems to have some favourable effect. Fourth, therapy targeting cellular factors

with a substance such as hydroxyurea may inhibit HIV replication. Hydroxyurea reduces ribonucleotides and inhibits DNA synthesis. It is widely used in medicine, crosses the blood-brain barrier and is relatively cheap.

Luc Montagnier discussed three approaches to AIDS research. The first is the study of apoptosis. All T-cell subsets are prone to apoptotic death and this correlates with CD4+ depletion. In vitro this apoptosis can be prevented with cytokines, especially IL-2. Whey protein also seemed to inhibit early cell death and requires more research. Secondly he spoke of oxidative stress and its relevance to AIDS pathogenesis. He said there was a decrease of glutathione in lymphocytes and an increase of peroxidized lipids and a drop in vitamin E serum level. Oxidative stress induces fast protein degradation, including a shorter life of IL-2. Finally he discussed the relationship between mycoplasma infection and HIV infection, noting an amazing affinity between the two. He concluded by saying that we need to move toward a global therapeutic approach in which antivirals, antibiotics, antioxidants and the restoration of the cytokine network are all applied simultaneously in an attempt to curb HIV replication.

Canada needs to prioritize and expand its research efforts. We conduct little basic science research. Our clinical research is based on the needs of pharmaceutical companies and often this research is nothing more than the addition of a few Canadian sites to an American trial. Better drug regimens which are affordable are needed so that people may live longer.

Access to treatments remains a very significant problem, especially to experimental therapies through the EDRP or through compassionate arms of trials. The biggest barrier to accessing approved treatments in most provinces remains the failure of governments to provide drug funding for all catastrophic diseases. Access to treatment based on one's ability to pay is unacceptable. Finally there is an urgent need to introduce viral load and resistance tests in a clinical setting immediately to measure on an individual basis the effectiveness of a given treatment.