J Biomed Sci 1995;2:1–11

HIV 10 Years Later – Where Do We Stand Now?

10th International AIDS Meeting, Yokohama

Lung-Ji Chang[a]
Yi-Ming A. Chen[b]
Mao-Yuan Chen[c]
Chen-Cheng Chou[d]
Shiing-Jer Twu[e]
Li-Min Huang[d]

[a] Glaxo Heritage Research Institute and Department of Medical Microbiology and Infectious Diseases, University of Alberta, Edmonton, Canada;
[b] Institute of Public Health, National Yang-Ming University,
[c] Department of Internal Medicine, College of Medicine, National Taiwan University,
[d] Department of Pediatrics, College of Medicine, National Taiwan University, and
[e] Institute of Public Health, National Taiwan University, Taipei, Taiwan, Republic of China

Abstract

A wide variety of up-to-date results and knowledge were presented at the 10th International AIDS Meeting, Yokohama. Epidemiologically, most interest was focused on the discovery of a new HIV subtype O, which cannot be reliably detected by currently available ELISA kits. Clinically, it is gradually appreciated that one single most important parameter is the viral load; the extent of viral load can help explain many clinical observations. Another eye-catching finding was the report of a clinical follow-up of a group of long-term nonprogressors. If the underlying operative mechanism can be elucidated, we can learn the necessary elements for halting HIV infection progression. Therapeutically, the trend has shifted to combination therapy, preferentially 3-drug combination of 2 RT inhibitors and 1 protease inhibitor. For the vaccine development, many novel vectors were introduced, but their potentials are unknown at present. The successful application of single-cell in situ PCR has changed our perception of HIV infection. This powerful technique can detect a single viral genome inside cells and revealed that a large proportion of cells already harbor HIV genomes soon after the entry of HIV into the body. A direct viral effect may fully explain subsequent T cell depletion without invoking a lot of indirect mechanisms such as apoptosis.

Key Words
HIV
AIDS
Epidemiology

Human immunodeficiency virus type 1 (HIV-1) was recognized to be the etiological agent for acquired immunodeficiency syndrome (AIDS) in 1984. Initially, it was felt that with increased knowledge about HIV scientists would cure this disease rapidly. However, 10 years hence, we are witnessing an alarming spread of AIDS with no parts of the world exempt from its devastating effect. Today we are far from the elimination of AIDS. Experience gathered over the last 10 years, nevertheless, does provide information for future directions of research.

The 10th anniversary conference on AIDS held in Yokohama in August 1994 has refocused the world's attention on AIDS as a global pandemic. This is the first time an Asian country served as host for this world AIDS conference. Seizing on the opportunity that many international AIDS experts were congregating in Asia for this meeting, several other satellite meetings were held in this region to coincide with the event. One such satellite meeting was in Taipei. This article is a progress report on the Yokohama meeting; another report on the Taipei meeting can be found elsewhere in this issue.

Received:
September 6, 1994
Accepted:
September 11, 1994

Li-Min Huang, MD, PhD
Department of Pediatrics
National Taiwan University Hospital
7 Chung-Shan South Road
Taipei, Taiwan 100 (Republic of China)

1021–7770/95/0021–0001
$8.00/0

Epidemiology

HIV Pandemic Worldwide and in Asia

To date, 4 million people have developed AIDS worldwide. 1.5 million did so just in the past year according to World Health Organization (WHO) estimates. Most cases (over 2.5 million) are concentrated in sub-Sahara Africa, which has the largest number of cases on a region-to-region basis. Alarmingly, this disease has spread rapidly in Southern and Southeastern Asia. The number of cases in all of Asia has increased from 30,000 to about 250,000 over the past year. The figure for Asia now represents 6% of the world total, compared with 1% a year ago. The total number of HIV-infected people in this region now stands at around 2.5 million, of whom about 40% are women. The number is predicted to rise 4-fold to over 10 million by the year 2000. According to a WHO projection, the annual incidence of HIV infections in Asia will exceed that in Africa in the not-so-distant future. Without prevention, analyses of McGraw-Hill estimate that AIDS will cost Asian economies between $ 38 and 52 billion in the rest of the 1990s.

It is estimated that implementing basic prevention programs in Asia would cost between US $ 770 million and 1.5 billion/year. This represents less than 0.03% of Asia's economic output. An investment of this kind could save an estimated 5 million infections by the year 2000. And the preventive effect would be even stronger as Asia moves into the 21st century.

Sex and HIV Transmission

Sexual transmission of HIV in Asia has been a serious problem in recent years. Seropositive rates jumped from 2 to 54% among commercial sex workers in Bombay from 1984 to 1992, and from 2 to 55% among intravenous drug users in Manipur from 1989 to 1990. In just 1 year, 1991–1992, seropositive individuals increased 10-fold among blood donors in Cambodia (from 0.076 to 0.75%) and almost tripled among intravenous drug users in northeast Malaysia (from 11 to 30%). It is evident that HIV is increasingly becoming a sexually transmitted disease, and rising heterosexual transmission is moving the pandemic from high-risk groups into the general population. In Thailand, HIV affects 16–72% of commercial sex workers, 6% of male sexually transmitted disease clinic patients, and as many as 20% of military recruits. The extent of HIV infiltration into the general population can also be seen in seroprevalence rates of 8% among antenatal women and 0.23% among blood donors.

Circumcision and HIV Transmission

Professor A. Ronald (University of Manitoba, Canada) presented a very high association between the presence of both the foreskin (uncircumcised penis) and genital ulcers and the rate of HIV seroconversion. The most persuasive data was from a 14-month prospective study that started with HIV-negative men. After 14 months, the largest numbers of HIV seroconverters were among the uncircumcised genital ulcer patients (48%) and the lowest among the circumcised nongenital ulcer group (2.6%). In between there were the uncircumcised no-ulcer group (26%) and the circumcised ulcer group (12%). This highly significant finding implicated the foreskin as an AIDS risk factor and is supported by an analysis of 28 studies, 26 of which suggested a strong correlation between HIV seroconversion and an uncircumcised penis. According to the combined data, the presence of a foreskin increases the risk of HIV infection 3-fold, and if one included a prospective 10-year period of usual exposure to HIV, circumcision would have the potential of reducing the seroconversion rate of a population at risk from 15 to 0.7% after 10 years.

Molecular Epidemiology of HIV-1 Subtypes O, E and H

Deducing from the variation of env and gag sequences, it has been demonstrated that nine different subtypes of HIV-1, A–H and O, account for HIV-1 infection in the world [6]. The global distribution of these genotypes shows that multiple genotypes may exist in different geographical locations. In one of the special 'recent report' sessions, HIV-1 subtype O viruses (the O stands for 'outlier' to emphasize the distance of each of these variants from known HIV-1 subtypes) which referred to 'newly identified' infections were reported by several experts. Although the first HIV-1 subtype O-Ant70 was identified and partially characterized in 1990 [1], it did not attract much attention until a recent report by a French group that some HIV-1 antibody tests used in blood banks may not reliably detect persons infected with the O virus. Most of the subtype O infections occur in persons from Cameroon or neighboring Gabon. Dr. L. Montagnier presented one case of an AIDS patient who died in 1992 from subtype O virus that could be documented to have been contracted before 1980. He suggested that subtype O has been present in the population from the start of the pandemic but simply had not been detected because its unusual antigenicity often presented as negative in standard EIA tests.

Dr. J.N. Nkengasong from the Institute of Tropical Medicine (Cameroon) reported that although the preva-

lence rate of HIV-1 infection in that country is less than 2%, among 18 isolates sequenced, 6 HIV-1 subtypes, A, F, B, E, H and O, could be identified. It was emphasized that, currently, subtype O is very rare outside of Cameroon. Dr. S. Mboup from Senegal reported that no anti-subtype O antibody reactivity has been detected in 470 persons, including 285 HIV-1-seropositive and 185 HIV-1-seronegative people from Senegal. The session raised concern about additional divergent HIV-1 subtypes, as yet unidentified, that might likewise be poorly detected by serological tests, especially those tests made of recombinant proteins or synthetic peptides rather than whole viral lysates (e.g., Clontech vs. Enzygost HIV-1/HIV-2 EIA).

Dr. J.R. George from the Centers for Disease Control and Prevention (CDC, USA) stressed the importance of systematic surveys for identifying new subtypes and assured the audience that the question of revamping the current HIV-1/2 screening tests to include subtype O antigens was under active consideration. One interesting point was that the amino acid hexamer sequences of the crown of the HIV-1 subtype O V3 domain have been found to be GPLAWY or GPMAWY, which might explain the negative results in those serological assays based on these regions.

Most of the information regarding HIV-1 transmission and pathogenesis to date has been obtained from North America and Europe, where subtype B is the predominant strain in the infected population. Since 1988, when explosive epidemics of HIV were documented in several Southern and Southeastern Asian countries, concerns about strain variation of HIV-1 isolates in Asia have increased greatly. Two years ago, at the Eight International Conference on AIDS held in Amsterdam, Dr. C.Y. Ou from CDC (USA) reported that two major types – A and B, which are equivalent to HIV-1 subtypes E and B, respectively – could be identified in different high-risk populations in Thailand. According to the published data, the subtype E was found mainly in female prostitutes and their clients in the Chiang Mai Province, while the subtype B was confined largely to intravenous drug users in Bangkok. However, little is known about the significance of the fact that the E subtype predominates in Thailand and perhaps other Southeast Asian countries.

At the Yokohama AIDS Conference, several reports focused on the sequence variation and phenotypic characteristics of subtype E. Dr. X. Yu from Johns Hopkins University reported that the HIV-1 subtype E viruses isolated in 1993 from AIDS patients in northern Thailand are significantly divergent form viruses characterized 2 years ago. Despite the predominance of GPGQ at the tip of the V3 domain in the previously isolated E subtype HIV-1 from 1991, the majority of the E subtype viruses isolated in 1993 had GPGR or GPGH sequences. In addition, rules for the prediction of syncytium-inducing (SI) versus non-syncytium-inducing (NSI) for B subtype HIV-1 applied poorly to the E subtype SI HIV-1.

Besides subtypes O and E, another new subtype H was reported in the epidemiology session. Dr. A. Bobkov from Moscow reported that the HIV-1 sequences isolated from southern Russia were highly divergent from other prototypic strains and resembled the subtype H isolate V1525 from Gabon.

Clinical Science

Currently, our basic knowledge of virus biology has not translated into full clinical usefulness. Therefore, for those infected with HIV-1, antiretroviral therapies are the most urgently needed and practically useful studies. However, there are problems with clinical therapeutics. For example, the antiretroviral agents now available result in moderately severe side effects, and their early clinical benefits disappear with the emergence of drug-resistant viruses. No major breakthrough has been achieved since the last international AIDS conference in Berlin. However some considerations for future treatment of HIV were outlined in Yokohama. Several new trends were encouraging, including the clinical trial of protease inhibitors, combination therapy to delay drug resistance, gene therapy, and immunomodulating agents.

Antiretroviral Monotherapy

The clinical benefits of zidovudine monotherapy has been tested in several double-blind placebo-controlled studies. Most of the results from these studies showed some short-term, but no long-lasting, efficacy. Interestingly, although the difference in survival rates was not statistically significant between immediate and deferred zidovudine treatment in the Concorde study, there was a clear persistent difference in CD4 cell counts between the two study groups. If the CD4 cell count was used as an end point, a significant difference would be noted in favor of immediate therapy. The clinical benefit of zidovudine seemed to be much clearer in preventing vertical transmission.

Maternal-to-Child HIV Transmission

Dr. Bryson of UCLA presented that rates of perinatal transmission varied, from 50% in Kenya to 25–30% in

the US to 13% in Europe. Of those infected 35% were infected before birth and 65% during birth. Despite uncertainties, we now understand that several factors influence the risk. These include maternal age, nutritional status and coinfections, conditions at delivery, and the presence or absence of certain HIV antibodies. A well-established link is that of risk of transmission and maternal viral load and immune status. Many markers of viral load are predictive of risk; moreover, CD4 cell counts correlate extremely well with risk: less than 200/mm^3 gives a 45% risk, over 500/mm^3 a 15% risk.

The overall analysis was that cesarian sections had a mildly beneficial effect. In a major French study, where conditions were maintained uniform, the transmission rate was far lower when cesarian sections was done with the 'water intact' than after the water had broken. The course of pediatric AIDS from a French prospective study of nearly 250 infected children was that 15–20% rapidly develop severe AIDS, most dying before age 4. But in 80–85%, the course is similar to adult AIDS, with the disease developing over a longer time period and a milder rate of encephalopathy. In the French study, 70% are alive at 6 years, more than $^1/_3$ of them with no signs of infections.

AZT Lowers Maternal-to-Child Transmission

Dr. Balsley (NIH, USA) reported on the recent and much-publicized study which showed that AZT reduced perinatal transmission of HIV. This study was a large cooperative study between hospitals in Europe and the US. Standard doses of AZT were given to HIV-positive women between 14 and 34 weeks of gestation, who had not been treated previously and had CD4 counts above 200. The treatment was extended to the newborns up to 6 weeks after birth. The results at 18 months were encouraging, showing a transmission rate of 25.5% in placebo cases compared to 8.3% in AZT-treated cases. This represented a 67.5% reduction in relative risk. Toxicity was low during treatment and up to the 18-month follow-up point. This important study proves that antiretroviral treatment during pregnancy could reduce rates of transmission.

Combination Therapy

Several reports showed that some patients on zidovudine could benefit by switching to didanosine monotherapy. However these studies were of short duration and the patient numbers were small. Dr. Graham from Johns Hopkins University presented data from the Multicenter AIDS Cohort Study (MACS), in which a comparison was made between combination antiretroviral therapy, switching to another monotherapy either with ddI or ddC, or continuing with zidovudine monotherapy. He concluded that the combination therapy was associated with significantly better survival compared to ddI or ddC alone, or continuing with zidovudine monotherapy. Since the absence of long-term efficacy and the rapid emergence of drug-resistant viral strains are two major drawbacks of monotherapy, sequential monotherapies would probably be replaced by combination antiretroviral therapy in the future.

There were a plethora of presentations on clinical trials of new antiretroviral agents. Many were protease inhibitors. Protease inhibitors, when used in combination with nucleoside-reverse transcriptase (RT) inhibitors, seemed to have incremental benefit of delaying the emergence of drug resistance. Most of these newer therapeutics are in early phases of clinical trial with safety and long-term efficacy results pending.

The battle against *Mycobacterium tuberculosis* was presented as a historical model illustrating the strategy for overcoming drug resistance. Streptomycin was the first effective drug for tuberculosis, to which *M. tuberculosis* quickly developed resistance. Later when other anti-TB drugs were available, it became evident that only a combination therapy could prevent the emergence of drug-resistant strains in successful treatment of TB patients. Applying this example to HIV-1 infection, zidovudine monotherapy beneficially impacted disease progression but lost clinical benefit after 2 years, presumably due to drug resistance. Combination therapy is expected to prevent or delay the emergence of resistance, thereby prolonging the clinical benefit. There were several reports of combination therapy. Dr. Julio S.G. Montaner from the Canadian HIV Trials Network presented results of combination therapy with AZT/ddI or AZT/ddC. Clinical benefits, in terms of an increase in CD4 cell counts, were noted with both regimens. The presentation by Dr. Mitsuya Hiroaki from the National Cancer Institute (USA) also showed increases in CD4 counts when patients were treated simultaneously with AZT/ddI. The serum viral load was reduced, but this did not correlate well with the increase in CD4 counts. His results raised the question of which prognostic markers are reliable. Interestingly, he observed that the combination therapy retarded the emergence of ddI-resistant mutation but not AZT-resistant mutation. If similar results were noted in other combination therapies with different formulas it would be of value to know the pattern of drug resistance. Dr. Van Leeuwen Remko from the University of Amsterdam described his results of adding AZT to 3TC monotherapy. Increases in CD4 cell counts and decreases in p24 antigen levels were observed. Dr. Ann C. Collier

(NIAID, USA) reported another interesting result showing that a combination of three antiretroviral agents was better than two agents (protease inhibitor Ro31-8959 + ZDV + ddC vs. Ro 31-8959 + ZDV or ZDV + ddC). Results of CD4 cell count and viral burden favored the simultaneous use of three agents. Combination therapy was generally well tolerated. However it remains to bee seen whether the clinical benefit will be long-standing and whether resistant strains occur.

Even if the combination therapy proved to be effective, a problem remains as to which is the right combination. Various combination have been tried. Dr. S. Vella from Instituto Superiore di Sanita (Italy) showed evidence that antiretroviral agents from a different class (Saquinavir plus AZT) when used in combination had the advantage of delaying resistance. It is certain that we will see many clinical trials of other combinations in the near future.

New immunomodulating agents were used in combination therapy from several new clinical trials. Dr. Chateauvert from the ZIDON study group (Canada) reported that combinations of α-interferon and zidovudine had no clinical advantage over zidovudine monotherapy. Dr. Jean-Marie Andrieu reported on the experience with corticosteroid treatment. Stabilization of viral load and increase of CD4 cell counts were noted after treatment with prednisolone for 1 year. Interestingly, in vitro experiment showed that glucocorticoid rescued CD4 T cells from HIV-1 activation-induced apoptosis. The initial results of clinical trials with interleukins were also reported; however, results were premature in terms of future potentials.

The Search for Surrogate Markers

Lack of predictive disease markers is one of the major impediments in evaluating antiretroviral treatments. Most current studies relie on CD4 cell count as the surrogate marker. This, unfortunately, is subject to variation and can only predict clinical benefits about one third of the time. At Yokohama, much emphasis was put on the viral burden to monitor disease progression. The quantitation of plasma viremia therefore was included in many clinical trials of new drugs in order to assess drug efficacy. Many speakers also discussed the phenotypes of virus isolates and tried to better correlate between clinical stages and the patient's virological status. At present a combination of CD4 T cell counts, quantitation of HIV-1 RNA and/or peripheral blood mononuclear cells (PBMC) proviral DNA, p24 antigenemia and clinical manifestations are used in most studies.

Vaccines and Immunology

The frightening pace of the worldwide spread of HIV demands that priority be palced on the development of an effective vaccine. Efforts have begun towards developing not only vaccines for uninfected individuals, but also therapeutic HIV vaccines, whereby HIV-infected individuals could be treated with immunogenes designed to boost salutary anti-HIV immune responses, decrease virus-infected cells, and either eradicate HIV or delay the onset of AIDS. There are many obstacles to the development of a successful prophylactic vaccine for the prevention of AIDS, including the lack of a known correlate of protective immunity, the genetic diversity of HIV, and the necessity to protect against transmission of the virus by either a parenteral or a mucosal route by either infected cells or free virions. A better understanding of the immune responses in HIV-infected people with different prognoses would probably uncover correlates of protective immunity that could guide the optimal construction of HIV vaccines. Much work remains to be accomplished in this area, especially in light of the fact that no truly adequate animal model for AIDS is available.

Long-Term Nonprogressor: Correlates of Protective Immunity against HIV

Because of a lack of an animal model of human AIDS and because a cohort of individuals naturally resistant to HIV infection is not available, the immune correlates of protection against HIV are not well known. However, the key to a better understanding of HIV immunopathogenesis and protective immunity might lie in the studies on long-term survivors whose immune response is unable to prevent establishment of infection but apparently prevents the development of AIDS. Dr. David D. Ho of the Aaron Diamond AIDS Research Center (New York, N.Y.) conducted a series of studies on a group of long-term nonprogressors. These nonprogressors are defined as individuals who have been seropositive with HIV for more than 12 years and yet have failed to develop the disease.

Ho reported that there were remarkably low levels of HIV in the blood of nonprogressors. With regard to the potential mechanism of enhanced control, his findings suggested that these individuals mounted particularly strong CD8+ cellular responses to HIV which suppressed viral replication. They also had markedly better neutralizing antibody responses. Furthermore, viruses isolated from these individuals were attenuated for replicative ability.

Although there are demonstrable neutralizing antibodies against HIV (and simian immune deficiency virus, SIV), albeit with reduced function in passive transfer experiments, most immunologists now consider cell-mediated immunity the most likely defense against HIV. Cytotoxic T lymphocyte (CTL) activity specific to the AIDS viruses has been found in peripheral blood lymphocytes, bronchoalveolar-lavage lymphocytes, lymph nodes, spleen, skin, and cerebrospinal fluid of HIV-infected persons and in animals infected with SIV. HIV infection in humans usually elicits a highly vigorous CTL response, and detailed maps of HIV-1-derived peptides recognized by defined HLA molecules are becoming available [5]. Furthermore, the replication of HIV in CD4 lymphocytes can be inhibited by CD8 cells with the phenotypic characteristics of CTL [8, 9]. It is therefore reasonable to expect that an effective HIV vaccine must stimulate HIV-specific cell-mediated immunity. One hypothesis [3] put forward in 1993, that received considerable interest and yet remains controversial, was that of a switch from a predominantly Th1 to a predominantly Th2 cytokine secretion pattern in the progression of HIV infection to AIDS. This hypothesis was examined by Jay Levy of the University of San Francisco (Calif., USA) who suggested strongly that the cytokine production profile of T lymphocytes from HIV-positive long-term surviving patients was characterized by a Th1>Th2 pattern. Further studies of the relationship between Th1 and Th2 cytokines and the ability of CD8+ T lymphocytes to suppress HIV replication in long-term survivors demonstrated a decrease of 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, which seemed to lead to disease progression. Suppression of CD8 activity by the transition of CD4 cells from the Th1 to Th2 type, therefore, leads to the onset of increased virus replication and the squelae associated with the progressions to AIDS. Thus it was suggested that approaches which maintain Th1 cell responses and/or CD8 antiviral activity may inhibit the virus and prevent the emergence of a virulent strain. However, Anthony Fauci, Director of the National Institute of Allergy and Infectious Diseases (USA) presented conflicting results. His group found that both cross-sectional and longitudinal analyses of constitutive cytokine expression did not demonstrate a switch from the Th1 to the Th2 phenotype during HIV disease progression in unfractionated or sorted CD4+ cell populations isolated from peripheral blood and lymph nodes. In addition, no evidence for a switch in the cytokine pattern was deteced after in vitro activation. Taking together, these results do not support the hypothesis that a switch from Th1 results in progression of HIV disease. In future experiments, it would be necessary to resolve the discrepancies in these cytokine data. It is extremely important to verify whether a Th1-like, cell-mediated, nonantibody response can control HIV. This would have enormous impacts for the design of vaccines and strategies for treating HIV-infected patients.

Clinical Trials of Candidate AIDS Vaccine in Humans

Animal models are needed to test the efficacy of strategies for eliciting protective immunity against HIV. Many important events in experimental model system for HIV vaccines took place in recent years. For example, the use of novel DNA vectors and immunologic adjuvants to achieve long-term antigen-producing capacity and to safely introduce CTLs [4], and the highly promising developments of protective immunity in primate models for AIDS [7] are some of the recent encouraging results. However, in spite of an extraordinary amount of work in the search of an animal model for human AIDS, no animal model that exactly mirrors human HIV infection has been established. In general, current animal models of HIV or SIV infections either do not develop AIDS symptoms or do not develop immune responses analogous to human anti-HIV T and B cell responses. Other problems include differences in model viruses and HIV-1 in viral sequences and in epitopes, failure to prevent infection by cell-associated HIV, uncertainties in safety of attenuated vaccines, and the need to use rare and expensive species such as chimpanzees. Hence, many important scientific questions pertaining to HIV vaccine development remain unanswered.

In contrast to the optimism about HIV-1 vaccines shown at the Berlin meeting last year, Dr. D. Bolognesi told the Yokohama conference delegates that several key features that have led to successful vaccines against other viruses appear to be missing for HIV. Due to breakthrough infections among HIV-1 vaccines (5 from NIAID trials and 1 from non-NIAID trials) reported recently, the NIAID AIDS Research Advisory Committee recommended that the institute continue to support, but not to expand, current vaccine trials with two gp120 subunit candidate vaccines. The decision was based on the following two reasons: firstly, if the efficacy of the vaccines were low or nonexistent, then an increase in risk behavior might occur, placing volunteers at higher risk for HIV-1 infection; secondly, if the vaccine facilitated infection or enhanced disease progression, the prospects would be even worse. Therefore, accessory issues have cast a cloud on the current HIV-1 vaccine efficacy trials in the United

States. Yet, the question remains whether the same issues would prevent vaccine development in developing countries with a more urgent HIV-1 problem.

The types of HIV immunogens currently being tested or being considered for testing in human clinical trials include inactivated HIV, protein subunit immunogens (individual HIV proteins such as gp120, gp160, or various of synthetic peptides of HIV proteins), multivalent HIV protein subunit immunogen mixtures, subunit immunogens in live vectors (Vaccinia, Salmonella, Galmette-Guérin bacillus, poliovirus, rhinovirus, or adenovirus, for example), anti-idiotypic antibody to CD4 or gp120, intracellular immunization (gene therapy), direct immunization with complementary DNAs of HIV proteins and immunization with host proteins (CD4 or MHC molecules). The advantages, disadvantages or concerns of these approaches and the current status of almost 30 clinical trials in human have been extensively reviewed [2, 3, 10]. In this meeting, HIV-1 or HIV-2 vaccine candidates using different vectors including canary pox virus (Alvac), live human adenovirus, live recombinant Mengo virus, recombinant Bacille bilié de Calmette-Guérin (BCG), chimeric SIVmac virus having HIV-1 *env* (SHIV) and live attenuated Salmonella bacteria were presented. Regarding immune responses, canary pox virus is rather benign, vaccinia is very strong and human adenovirus seems to be intermediate.

To increase or broaden the immune response to envelope proteins and peptides, trials were done to compare the same immunogen in combination with more than one adjuvant. Dr. J.W. Eichberg (USA) reported on the efficacy of live human adenovirus-HIVMN env recombinant vaccines (Ad-gp160MN) followed by either homologous (MN) or heterologous (SF2) subunit boosts in dogs and chimpanzees. The results showed that Ad-gp160MN elicited a neutralizing antibody and CTL immune responses to the HIVMN envelope. A broadening of the neutralizing response was observed by heterologous subunit boosting. Upon challenge by virus inoculation, none of the animals had any detectable virus signal, as determined by PCR and serology up to 12 weeks after challenge. However, no single immune parameter correlated with protection. The combination of rgp160 with either a recombinant 'live' vector (canarypox-gp160) or with a V3 peptide sequentially expands the knowledge derived from earlier combinations of recombinant vaccinia with rgp160. Another trial has begun to examine the various recombinant evelope proteins as boosts following priming with vaccinia-gp160.

Regarding humoral immunity against recombinant protein vaccine candidates, neutralizing antibodies could be generated after immunization; however, antienvelope responses last for several months, rather than years, and reimmunization has not yet been shown to maintain neutralizing antibody levels. In addition, although quite effective in their ability to induce neutralizing antibodies to several laboratory isolates, the immune reponses elicited by these vaccines have failed to neutralize fresh patient isolates. As for cell-mediated immunity to HIV candidate vaccines, in contrast to what is observed in HIV-1 infected people, CTL have not been detected in the blood of vaccinated volunteers when tested directly. CTL precursors have been demonstrated in one third of the PBMC of volunteers immunized with a recombinant canary poxvirus HIV candidate vaccine, with boosts of recombinant gp160. In view of heightened interest in Th1 responses and the potential role of cytokines in viral suppression, the significance of immunologic memory, as reflected by lymphoproliferation and secretion of cytokines, needs to be further assessed.

Dr. M. Honda from Japan demonstrated that a recombinant BCG vector containing an HIV-V3 insert can effectively induce B cell and T cell immune reponses. Because of the vast number of BCG vaccinations given over the past 40 years, there are excellent data indicating that the incidence of serious side effects is relatively small. However, Dr. D. Bolognesi reminded researchers and the audience that it may be a drawback to use BCG-HIV vaccine for those countries that include BCG in their routine vaccination program. Another interesting vector with great application potential is Salmonella, since it can be given orally. Dr. Y. Lu (USA) reported that a live attenuated *Salmonella typhimurium* strain SL3261 expressing HIV-1 antigen was constructed by autologous recombination. Mice were immunized orally by a single dose of 10^8 salmonella bacteria. The resulting B cell and T cell immune responses were quite impressive.

Basic Science

An entire decade has passed without a clear solution to the 20th century disease caused by HIV. Nevertheless, the mysterious course of the development of the disease has been gradually revealed through the laborious study of its immunopathogenic mechanisms. This topic was covered comprehensively by Dr. Anthony Fauci in his recent studies of HIV-caused immunopathogenicity. Fauci suggested that the disease progress is critically dependent on early

events which occur soon after the initial infection. In the early stage of infection, an acute viremia leads to the trapping of HIV in the lymph nodes. What is important is that the response of CD8+ T cells which may be protective or harmful throughout viral latency. The so-called 'inappropriate' cytokine responses, Vβ subset (e.g. Vβ14 and Vβ19) expansions, and other host factors all play a part in the destruction of the lymphatic structure. During the advanced stage of disease, the trapped virus is shown to spread to the peripheral blood and tissues. The message is clear – treatment for HIV infection must be both early and effective.

Ashley Haase (USA) presented a single cell in situ PCR technique that showed that the virus reservoir in the lymphoid tissues is much larger than previously thought. This study measured the dynamics of immune depletion during HIV infection. During latency, he found fewer than 5 copies of RNA per infected cell. His technique uses two pairs of PCR primers with a short 2 bp overlap that was sensitive enough to detect a single copy of integrated DNA. Using a double staining method which stained CD4 cells first (brown) then PCR amplified HIV DNA (green), this technique allowed for the identification of CD4 cells infected in different lymphoid compartments. Haase showed data indicating that there is a large number of covertly infected cells in HIV patients at the early asymptomatic stage. Almost 25% CD4+ cells harbored HIV DNA, but less than 1% of them were HIV RNA+, which would indicate productive infection. This in situ amplification method detected more HIV+ cells in the CD4+ pericortical regions of lymphoid tissues. Some CD4+ cells were negative in the germinal center; however, in the spleen, most CD4+ cells were also HIV+. In fixed tissue macrophages, most cells harbored HIV but no RNA was detected. This study provided direct evidence that there is a large intracellular source of HIV and also confirmed that there is an extracellular source of HIV associated with the dendritic cells. Haase suggested that there is a slow conversion of DNA– to DNA+ cells. During the dormant state, the provirus persistently spreads into a large reservoir including macrophages and lymphoid tissues, and activation of latent provirus is an ongoing process which over time results in a large pool of productively infected cells with depletion of CD4+ lymphocytes and eventually development of disease.

T cell stimulation is mediated through an MHC-peptide interacting with TCR-α and TCR-β with CD3 and CD4 molecules. To proliferate, T cells need a costimulation signal that interacts with the surface CD28 and CTLA-4 molecules. This is mediated by B7-1 (CD80) and B7-2 (CD86) on antigen-presenting cells. Haffar et al. of Bristol-Myers Squibb (USA) demonstrated that HIV LTR was activated when T cells were stimulated with anti-CD3 and anti-CD28. The HIV activation was moderate when anti-CD3 and IL2 were used, indicating that the CD28 engagement was not solely due to IL-2 production. The CD28-mediated effect was strengthened by the fact that soluble CTLA-4 Ig (1 μg/ml) efficiently inhibited 90% of the stimulation.

One highlight of the 10th anniversary AIDS meeting was a look at nonconventional treatments for HIV infection. Several speakers presented works using genetic approaches to combat the virus. Robert Gallo (USA) presented several approaches including antisense oligo inhibition, gene therapy by TAR decoy, dominant negative Rev, anti-RT, anti-Gag single chain Fv, and hydroxyurea therapy. The antisense oligo GEM91 (directed against Gag) is currently being used in clinical trials in France and the US. Dr. Gallo illustrated an efficient anti-HIV effect (near 90%) using anti-RT intracellular single chain antibody directed against various strains of HIV.

The study of HHV-7 in Dr. Gallo's group has identified that CD4 is also used as a receptor for HHV-7, and it appears that HHV-7 infection of CD4 blocks HIV replication. Currently the Gallo group is investigating which polypeptide of HHV-7 is responsible for this effect. Additional evidence that CD4 is the receptor for HHV-7 was presented by Paolo Lusso. He showed that HHV-7 infection was blocked by monoclonal antibody to CD4, soluble CD4 and soluble HIV gp120. Also, CD4-negative cells became susceptible to HHV-7 infection after they were transduced with a CD4 gene.

Mario Stevenson (USA) discussed HIV integration in nondividing cells. The HIV preintegration complex contained both Gag matrix protein (MA) and the Vpr protein. Both contain a nuclear localization signal. The mutagenesis study showed that mutations in either MA-NLS (nuclear localization signal) or Vpr alone do not affect HIV replication in human peripheral blood lymphocytes. In primary macrophages and nondividing cells the wild type virus replicated well; mutations in either MA-NLS or Vpr reduced the replication rate; and mutations in both MA-NLS and Vpr abrogated viral replication totally.

Matija Peterlin (USA) used a CD8-Nef fusion construct to study Nef function. Expression of CD8-Nef in Jurkat cells activated NF-kB expression and induced cell death by apoptosis. The recovered survivors carried truncated *nef*. Precipitation of CD8-Nef with anti-CD8 antibody brought down a cellular protein of 62–72 kD, which did not associate with the truncated CD8-Nef. This study

showed that Nef causes persistent activation of T cells by membrane association and signaling transcription.

Donald Mosier (USA) used a scid-hu-PBL model to study the mechanism of CD4 cell depletion. His group showed that macrophage-tropic (SF162) virus-infected cells tend to induce apoptosis of nearby uninfected CD4+ cell, an effect not observed for the T cell-tropic (SF33) virus. The T cell-tropic virus induces cell death of the infected cells but not the bystander cells. Fast CD4 cell depletion was observed when cell-asssociated SF33 virus was used and slow CD4 cell depletion was observed when cell-free SF33 was used. The Mosier group suggested that macrophage tropic virus and a high local concentration of T cell-tropic virus induced killing of bystander cells and rapid depletion of CD4 cells.

CD26 was reported to be the coreceptor of HIV by Ara Hovanessian from the Institut Pasteur. At this meeting he suggested that CD26 is a cofactor (not a 'receptor') for HIV infection. CD26 is a dipeptidyl peptidase IV (DPP IV). Peptidyl inhibitors (e.g. KPR, IPI) at 5.6 m*M* inhibited DPP IV activity and blocked HIV entry. The HIV interacting site on CD26 is near the N terminus of CD26 and Dr. Hovanessian found that the interaction was blocked by peptide inhibitors. When the peptide inhibitor [5(KPR)TASS] was added at 5 μ*M*, 10 min prior to HIV addition, more than 90% of inhibition was observed. Syncytium formation was totally inhibited at 50 μ*M* concentration. He suggested that the inhibition of DPP IV peptidase activity in CD26 is not essential to this effect. Instead, the anti-HIV effect is due to inhibition of apoptosis, syncytium and entry. Somewhat controversial results were presented by A. Dolei (Italy). Study by that group of HIV binding and replication using human mesenchymal and epithelial cells indicated that although CD26 was expressed on all these cells, there was no correlation between CD26 expression with HIV binding or syncytium formation. However, they showed that surface CD44 was downregulated while CD26 expression was enhanced in HIV-infected cells.

Jaap Goudsmit from Amsterdam indicated that almost all new HIV infections are initiated by NSI rather than SI virus and the antibodies induced by NSI virus are usually not of the neutralizing type. SI virus induces lower antibody activity but is of the neutralizing type. NSI virus grows fast and SI virus grows slowly, and 90% of AIDS patients developed diseases with either NSI virus or NSI-SI mixture. The *env* mutations seem to play an important role in the process of HIV pathogenesis. Dr. Goudsmit pointed out that the high SI virus load, rapid CD4 decline and disease progression, characteristic of AIDS, were often caused by the escape *env* mutants of NSI virus.

The pathogenesis of the Kaposi sarcoma (KS) in AIDS patients was investigated in two talks. Robert Gallo described the KS cells as spindle cells from detached endothelial cells. KS can be induced by fibroblast growth factor (FGF), where Tat acts like fibronectin to assist the detached endothelial cells to attach. HIV infection induces an expression of inflammatory cytokine and FGF, and promotes KS growth. A supporting view presented by Buonaguro et al. showed that Tat can induce tumor necrosis factor-β (TNF-β) expression by binding to a TAR-like element at +10 to +115 in the TNF-β gene and interacting with upstream NF-κB and Sp1 elements, thus inducing KS growth.

Furuta et al. from Kyoto University (Japan) presented results showing that the *cis* regulatory element of Rev is not absolutely important for Rev function, in the case of cytoplasmic RNA expression. This group studied a series of constructs containing different regions of *gag, pol* and *env* +/– RRE and showed that the splicing signal and possibly the secondary structure are more important for Rev function. Experiments undertaken by Pavlakis et al. showed that the removal of instability sequences in the p17 *gag* region allowed the expression of Gag proteins and assembled particles in mouse cells where Gag expression was normally blocked due to Rev defect. This replacement also generated RRE-minus and Rev-minus replication-competent virus in PBMC.

HIV replication independent of Tat has been established in long-term culture. The work presented by L.-J. Chang (Canada) demonstrated that high titers of *tat*-minus virus were produced in several cell lines infected with *tat* mutants containing triple stop-codon or deletion mutation in the *tat* gene. Studies of this culture system indicate a second function of Tat in the enhancement of Gag processing. The establishment of *tat*-minus HIV high producer cell lines may prove to be useful for a whole inactivated or the live attenuated viral vaccine development.

Philip Greenberg from University of Washington presented a series of gene therapy strategies including adoptive immunotherapy using CD8+ CTL, genetic modification of effector T cells, and intracellular immunization. Clinical trials using cytomegalovirus (CMV)-specific CTL to treat patients showed promising results with 0 out of 13 developing CMV disease. However, there are potential risks for HIV patients with such treatment including the development of pulmonary edema and meningoencephalitis. Therefore there is a requirement for eliminating

the chronic persistence of effector T cells. The method Dr. Greenberg proposed was to introduce a hygromycin-HSVtk fusion gene into the therapeutic T cells by retroviral infection. This fusion protein exhibited resistance to hygromycin and was sensitive to ganciclovir. So far, 3 patients have been treated with *gag*-specific CD8+ cell clones and the T-cell-related toxicity is controlled by ganciclovir. His strategy for genetic modification of effector T cells was to induce high IL-2 expression to enhance CTL (Th1) response. Activated CD8+ cells secrete many different cytokines but not IL-2 which is normally secreted by CD4 cells. Two approaches can be used to induce IL-2 expression; one is to transduce T cells with an interferon-γ-driven IL-2 gene and the other is to fuse the granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor (extracellular domain) and the IL-2 receptor (intracellular domain) in one molecule so that this heterodimeric chain will respond to GM-CSF (10 ng/ml) and induce IL-2 expression. In the intracellular immunization strategy, Dr. Greenberg presented five different approaches for reconstituting CD4 T cell immunity, including the use of 25 TAR decoy elements plus antisense gene, the use of RRE decoy, M10 Rev *trans*-dominant mutant, double hammerhead ribozyme targeted to *tat* gene and hairpin ribozyme targeted to the U5 region. Preliminary studies have shown promising results with treated CD4+ T cells surviving HIV infection for longer than 10 weeks.

HIV LTR can be activated by several heterologous viral *trans*-activators such as HSV ICP4, HCMV IE1 and IE2, and HBV X. Kim et al. from the Seoul National University (Korea) showed that HIV LTR can be induced by HCMV IE2 up to 45-fold but this effect was suppressed by the retinoblastoma gene product (down to 4-fold). The study of NF-κB-and Sp1-deleted HIV LTR indicated that the IE2-mediated *trans*-activation is independent of these transcription elements. The suppressive effect of the RB protein is not dependent on κB and Sp1 elements and is seen also with other promoters such as SV40.

Ishii et al. (Japan) have identified a new class of factors called EP1, EP2 and EP3 that bind to HIV LTR enhancer NF-κB sites. The high basal level of expression of EP2 in T cells can be further induced by PHA and PMA. In addition, a new factor called HIV-TF1 was identified which binds 60 bp 5′ to the κB sites and assists the NF-κB binding. The Ishii group also demonstrated that c-*myb* binds to HIV LTR (–304 to –297 and –113 to –120) and activates transcription.

Dr. Hatanaka (Japan) presented studies on acutely and chronically infected cells and illustrated that HIV infection destroys the nucleolus structure in acute but not chronic infection. It turned out that the destructive effect was caused by the Rev protein. Using confocal laser-scanning microscopy, Dr. Hatanaka showed pictures of enlarged, deformed nucleoli in infected T cells which was different from the shrinking characteristics of apoptotic cell death. By overexpression of Rev in Cos cells, induced expression of Rev in hamster cells, Dr. Hatanaka illustrated an universal cell killing effect of Rev in human, monkey and hamster cells. The region that mediates this function was reported in an arginine-rich domain between amino acid 36–50 in Rev. This region specifies pleiotropic functions including RRE binding, nucleolar targeting and multimerization. From this study, his group generated a transdominant Rev mutant (deletion of a.a. 38–44). This transdominant mutant when transduced into CD4 HeLa cells and T cells protected cells from the killing effect of HIV infection. The currently approved version of transdominant M10 Rev in clinical trials was shown to be cytotoxic like the wild-type Rev in Dr. Hatanaka's study. Thus, the newly generated Rev transdominant mutant should be better than the M10 Rev mutant in gene therapy trials.

Concluding Thoughts

Although 10 years have elapsed and we still have no solution for AIDS, studies on HIV have provided important scientific findings. In some cases (as in the long-term nonprogressors) natural immunity seems to control the virus. If we could clarify the immune mechanisms operative in these long-term nonprogressor, it may be one of the most important discovery to emerge from HIV research. We have also learned that AIDS, like other infectious diseases such as tuberculosis, requires a combination of multiple drugs. By eliminating or delaying the emergence of a resistant strain, one could conceive of prolonging the disease-free course. While our current knowledge is insufficient to adequately treat the disease, the lessons that we have learned have contributed to our understanding of viral infections and antiviral therapies. Perhaps, we will not need another 10 years for a successful treatment of this pandemic.

Acknowledgments

We thank Dr. Che-Yen Chuang for helpful discussions and Dr. Cathy Fletcher for help with the preparation of the manuscript.

References

1 De Leys R, Vanderborght B, van den Haesevelde M, et al. Isolation and partial characterization of an unusual human immunodeficiency retrovirus from two persons of west-central African origin. J Virol 64:1207–1216;1990.

2 Haynes BF. Scientific and social issues of human immunodeficiency virus vaccine development. Science 260:1279–1286;1993.

3 Letvin NL. Vaccines against human immunodeficiency virus – progress and prospects. N Engl J Med 329:1400–1405;1993.

4 Liu MA, Vogel FR. Use of novel DNA vectors and immunologic adjuvants in HIV vaccine development. AIDS 8(suppl 1):S195–S201; 1984.

5 McMichael AJ, Walker BD. Cytotoxic T lymphocyte epitopes. Implication for HIV vaccine. AIDS 8(suppl):S155–S173;1994.

6 Myers G, Korber B, Berzofsky JA, Smith RF, Pavlakis GN (eds). Human Retroviruses and AIDS; a Compilation and Analysis of Nucleic Acid and Amino Acid Sequences. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, New Mexico, 1993.

7 Schultz AM, Stott EJ. Primate models for AIDS vaccines. AIDS 8(suppl 1):S213–S236; 1994.

8 Tsubota H, Lord CI, Watkins DI, Morimoto C, Letvin NL. A cytotoxic T lymphocyte inhibits acquired immunodeficiency syndrome virus replication in peripheral blood lymphocytes. J Exp Med 169:1421–1434;1987.

9 Walker CM, Moody DJ, Stites DP, Levy JA. CD8+ lymphocytes can control HIV infection in vitro by suppressing virus replication. Science 234:1563–1566;1986.

10 Walker MC, Fast PE. Clinical trials of candidate AIDS vaccines. AIDS 8(suppl 1):S213–S236;1994.