HIV VACCINE DEVELOPMENT: A SUBTYPE E-SPECIFIC STRATEGY

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Abstract. The pandemic of HIV/AIDS consists of multiple foci with distinct epidemiological characteristics. Among the approximately one million Southeast Asians infected with HIV, subtype (clade) E infections predominate. This subtype, a recombinant virus comprised of a clade A core (*gag*) gene and a mosaic clade A/clade E envelope (*env*) gene, became broadly epidemic in Thailand beginning in 1989. Since then, subtype E HIV has become increasingly prevalent throughout Southeast Asia.

Consistent with the recent introduction of clade E HIV, the diversity of Southeast Asian subtype E viruses is narrow (6% nucleotide diversity across *env*). Since neutralizing antibodies may play a protective role against HIV infection, and are relatively clade specific for genotype E viruses, a subtype E-derived candidate vaccine tested in Southeast Asia would provide an optimal test of vaccine concept. It would also provide, for the first time to a developing region of the world, a non-B clade candidate vaccine designed specifically for the local epidemic.

A consortium of industry (Chiron Vaccines and Pasteur Merieux Connaught), academic (Mahidol and Chiang Mai Universities) and military (United States and Royal Thai Army Medical Departments) medicine is working together to develop and test HIV vaccines for the genotype E epidemic. A genotype B recombinant glycoprotein (rgp)120 candidate vaccine has undergone phase I/II testing in Thailand and confirmed to be safe and immunogenic in this ethnic group. An rgp120 (E) has been produced and a phase I/II trial of the bivalent product (B/E) is in the final stages of approval. This vaccine construct is designed to elicit humoral immune responses. To augment these antibody responses with CD8+ CTL responses, an E-specific, live-vectored vaccine is being developed which will be used in conjunction with rgp120 in a second vaccine approach. Canarypox (ALVAC) constructs containing multiple HIV genes (*gag/pol/env*) currently designed for the subtype B epidemics will be modified to contain a clade E *env* gene sequence.

After predetermined milestones have been met, these two subtype E-specific candidate vaccines will be assessed for protection in a large collaborative efficacy trial. Since neither animal models nor laboratory assays are validated as predictive of HIV vaccine efficacy, it must be through such a phase III trial that vaccine-induced protection and immunologic correlates will be determined.

HIV SUBTYPES

Study of HIV has shown that this virus is characterized by genetic diversity. Within an individual, this diversity is manifest as a quasispecies with innumerable closely related viruses making up a population. In contrast, at the global level viruses have been found to form genetic clusters (clades), divergent from each other. The meaning of these genetic clades or subtypes is not fully determined. Subtyping has already proved invaluable to the epidemiologist, even allowing the distinction of separate epidemics within a country as was the case in Thailand (Weniger *et al,* 1994). Beyond this, genetic subtypes may correlate with biological properties such as virus neutralization. Protection from HIV-1 infection induced by a subtype B rgp160 vaccine candidate was demonstrated against an intraclade challenge (Girard *et al,* 1995) but not against a subtype E (interclade) challenge (Girard *et al,* 1996). Most importantly, it is not known whether or not the breadth of efficacy of an HIV vaccine will be limited by subtype differences and thus require a multivalent vaccine to be globally useful.

HIV sequence data, both genetic and protein, has been used to develop a phylogenetic classification system. An HIV sequence database is maintained at, and made available from, the Los Alamos National Laboratory in the United States (Leitner, 1996). The vast majority of HIV isolates fall in the M (main) Group, with a small number in the O (outlier) Group. Viruses of the M Group have been found to cluster into clades which have been given subtype names from A to J. These subtypes are of approximately equal genetic distance from each

other and no strain has been found which would represent the center of this star phylogeny. The envelope glycoprotein (gp120) differs by approximately 30% between genotypes, with as much as 15% diversity within genotypes (Louwagie *et al,* 1995).

Subtype A, found mainly in Central Africa, is the most genetically diverse subtype. Subtype B is the most studied, due to it's position as the most prevalent subtype in the western world, and is the basis for most HIV diagnostics and candidate vaccines thus far. Subtype C is of special importance in Asia because of its high prevalence in India, with introductions into Malaysia (Brown *et al,* 1996). Subtype E deserves special attention and is the focus of vaccine strategy in Southeast Asia. This was the first non-B HIV virus described (McCutchan *et al,* 1992), suggesting the existence of multiple subtypes. Subtype E viruses have been shown to be recombinants of a subtype A virus (*gag* and 3′ half of gp41) with an ancestral E virus (5′ half of gp41 and gp120) (Leitner, 1996). Sequencing of the full 10 kilobase genome of a subtype E virus from Thailand revealed that it was a mosaic with multiple crossovers in its A-E recombination (Carr *et al,* 1996).

The HIV subtype E viruses of Southeast Asia were initially found to be more tightly clustered (less diverse) than those of the other subtypes, as assessed in viruses from both Thailand (McCutchan *et al,* 1992) and Cambodia (Artenstein *et al,* 1995; Porter *et al,* 1997). Compared with virus from SE Asia, subtype E viruses from central Africa are more than three times as diverse in their *env* gene (McCutchan *et al,* 1996). While the viruses isolated from individuals with Southeast Asian acquired HIV infections who are asymptomatic have a mean difference in their envelope gene (C2-V5) of 6.6%, those from patients with AIDS have a difference of 12% (Yu *et al,* 1995; McCutchan *et al,* 1996). Since the vast majority of infected individuals in Southeast Asia are still asymptomatic, ongoing transmission in the near term is expected to reflect the narrower diversity of the early-moderate stage infections.

HIV MOLECULAR EPIDEMIOLOGY IN SOUTHEAST ASIA

In 1996 the World Health Organization estimated that 5.2 million people were living with HIV/AIDS in South and Southeast Asia (WHO, 1996). While this number made up 23% of the global pandemic, it was estimated that only 6% of all AIDS cases had occurred in this region. The comparatively high ratio of people infected with HIV to those with late-stage disease reflects the later introduction of the virus in this region than in Africa.

The global HIV pandemic is made up of multiple regional and more focal epidemics. In addition, within given areas different viral variants have been introduced and spread through human networks with distinct risk factors. Data available from Southeast Asia, point to HIV subtype E as being the predominant variant with subtype B also circulating in particular social networks and population groups.

In Thailand, two distinct epidemics of HIV apparently began in the late 1980s (Weniger *et al,* 1994). An epidemic of sexually transmitted HIV developed in northern Thailand while a separate epidemic of parenterally transmitted infections began among injecting drug users (IDUs) in Bangkok. Viruses isolated from these two epidemiological groups were found to be phylogenetically distinct (McCutchan *et al,* 1992; Ou *et al,* 1993). The northern Thai and IDU isolates each clustered tightly but separately. In the typing system based on the envelope gene, they were found to be subtype E and B viruses, respectively. In years since then, subtype E virus infections have also been introduced into and spread within the IDU population. Wasi and colleagues (1995) found that the proportion of HIV subtype E infections among newly infected Bangkok IDUs increased from 3% in 1988-1989 to 44% in 1992-1993. The wider epidemic in Thailand is sexually transmitted and ongoing surveillance of 21-year-old, male military conscripts shows that serotype E infections make up more than 90% of prevalent HIV cases (Markowitz, personal communication).

The molecular epidemiology of HIV in other countries of Southeast Asia has been studied in less detail than in Thailand. Nonetheless, subtype E strains are apparently widespread and often appear predominant. Within Myanmar, subtypes B and E seem to have risk associations similar to those in Thailand (Takebe *et al,* 1996). Subtype B was found in IDUs, E in commercial sex workers (CSWs) and patients with sexually transmitted diseases from the eastern and southern parts of the country while both subtypes were found in Yangon. Two small

studies of the viruses infecting south Vietnamese found subtype E in both IDU and CSW groups (Menu *et al,* 1996; Nerurkar *et al,* 1996).

As of the end of 1996, only 235 cases of AIDS had been reported from Cambodia (WHO, 1996) but a recent severe epidemic appears to be occurring. HIV infections acquired in 1992 by military participants of the United Nations Transitional Authority Cambodia (UNTAC) were found to be caused by subtype E viruses (Artenstein *et al,* 1995; Soeprapto *et al,* 1995). In Malaysia, HIV from IDUs collected in 1992-1993 were mostly subtype B; subtype C viruses were also identified in two individuals who had received transfusion or organ transplant in India (Brown *et al,* 1996). A study based on serotyping has found subtype E to be predominant among CSWs and B among IDUs (Beyrer, personal communication). Indonesia reportedly also has both subtype B and E viruses in circulation, although most of the E viruses have been obtained from soldiers who had participated in UNTAC (Porter *et al,* 1997). From Lao PDR, where only 17 cases of AIDS had been reported as of 1996 (WHO, 1996), no data on HIV subtype is available.

SERUM NEUTRALIZATION OF HIV

While the correlates of immune protection against HIV remain unknown, neutralizing antibodies are thought to play a key role in reducing the infectivity of free virus particles. Results of neutralization assays are very dependent on technical variables, and biological relevance has not been validated by correlation to protection in humans. While antibodies elicited by current candidate vaccines neutralize laboratory-adapted HIV of the same clade (homologous virus) (Mascola *et al,* 1996), their capacity to neutralize primary isolates appears further limited, to viral isolates having envelope glycoproteins very closely related to the immunizing strains (Zolla-Pazner *et al,* 1997). This latter recent finding is important in suggesting that neutralization function is indeed inducible by vaccines, though breadth and magnitude may need enhancement.

The narrowness of the diversity of gp120 in the Southeast Asian subtype E viruses suggests this to be an ideal target for a subtype-specific vaccine designed to elicit neutralizing antibodies. Mascola and colleagues have shown that in the case of subtype E and B viruses, the genotype predicts neutralization serotype (Mascola *et al,* 1994, 1996). This correlation is reported to not hold across the other genotypes (Kostrikis *et al,* 1996), and may be related to the unique homogeneity of subtype E viruses in Asia.

SUBTYPE E-SPECIFIC VACCINE STRATEGY

As genetic studies revealed that the HIV pandemic was based upon multiple subtypes of virus, it became clear that vaccines based upon subtype B viruses might not be globally protective. Thus, researchers at the Walter Reed Army Institute of Research (WRAIR) offered their well characterized subtype E viruses to vaccine manufacturers and encouraged use of these in candidate vaccine design. Chiron Biocine (now Chiron Vaccines) had already made an rgp120 product in mammalian (CHO) cells derived from SF2, a subtype B virus. This corporation agreed to develop a similar product with one of the subtype E viruses (CM235) if medical leaders in Thailand agreed to carry out clinical studies of the product (Duliege *et al,* 1996). With the goal of bringing a subtype-specific candidate vaccine to one of the major non-B epidemics of the world, an international collaboration has been established bringing together military medicine, academia and industry. The US and Royal Thai Armies built upon their 35-year-old association at the Armed Forces Research Institute of Medical Sciences with experience in field studies, vaccine trials and laboratory sophistication. The initial university partner was the Research Institute for Health Sciences, Chiang Mai University, expanding in 1996 to include both the Vaccine Trial Center and Siriraj Hospital of Mahidol University. Industrial partnership has expanded from Chiron Vaccines to also include Pasteur Merieux Connaught.

This consortium, called the Thai AIDS Vaccine Evaluation Group (TAVEG), has designed a strategy for HIV vaccine development which is subtype specific and considers the several immunological mechanisms upon which vaccine efficacy might be based. Challenges to HIV vaccine design are great, including both cell-free and intracellular localization, high rates of genetic variation based upon both mutation and recombination, and the multi-year duration of an asymptomatic infectious state.

The TAVEG proposes following two parallel paths, one based upon the humoral and the other the cellular arms of the immune system. Pathway A is intended to develop a vaccine which induces antibodies capable of neutralizing cell-free viral particles. This is the mode of action of soluble recombinant proteins. Thus, the Chiron rgp120 candidate vaccine is being developed to test this concept. A phase I/II trial of the subtype B-based rgp120 vaccine was carried out by the TAVEG in 1995-1996. The vaccine was shown to be safe and immunogenic in this population, just as it was in North Americans (Graham *et al,* 1996). The subtype E-based rgp120 has been manufactured under an IND from the US Food and Drug Administration and a 380-subject, phase I/II trial is planned to start in the last quarters of 1997. This trial, to be carried out at four sites in two regions of Thailand, will assess safety, subtype-specific and cross immunogenicity, and dosage.

Pathway B of the TAVEG's strategy is aimed at evaluating a vaccine which elicits CD8 cytotoxic T lymphocytes (CTL) which are HIV specific. The candidate vaccine chosen to represent this immunologic concept is the ALVAC strain of the canarypox virus (Perkins *et al,* 1995) genetically engineered by Pasteur to carry and express HIV genes (Fleury *et al,* 1996). This live vector expresses HIV gene products intracellularly, allowing their presentation in the context of MHC class I molecules. Safety of this avian poxvirus is insured because of its inability to replicate in humans (Baxby and Paoletti, 1992; Plotkin *et al,* 1995). The current ALVAC product (vCP205) carries *gag/pol/env* genes derived from subtype B viral strains and elicits CTLs, some of which cross-react against subtype E viruses (Ferrari *et al,* 1997). After first testing this product for safety and immunogenicity in Thai subjects, the *env* gene will be replaced by a subtype E gene sequence for subtype-specific evaluation in Thailand. (The *gag/pol* genes will not be modified because of their greater conservation across subtypes.) This ALVAC candidate vaccine will be given in combination with the Chiron rgp120 product, often referred to as "prime-boost", with the goal of eliciting both CTL and neutralizing antibody responses.

Since the immunological mechanism(s) by which an HIV vaccine may protect is not known, the TAVEG believes it essential to pursue both pathways of vaccine development in parallel. While the "prime-boost" concept may appear more attractive since it is aimed at eliciting both arms of the immune system, it will be the more costly vaccine to produce and more demanding to provide to populations in need with its more stringent cold chain requirements. Thus, for optimal public health benefit it is important to determine the efficacy of both types of candidate vaccine.

Following these parallel pathways, the two candidate vaccines will be assessed against predetermined milestones: safety, immunogenicity of the type expected with durability of response and boosting effect, and stable formulation. If the milestones are met, the TAVEG will plan with the National AIDS Commission of Thailand for phase III testing. This will be designed as a three-arm efficacy trial, assessing the vaccine of each pathway with that of a placebo. The measure of success of such an important field trial will be whether a statistically sound answer can be reached as to whether either candidate vaccine is efficacious. Thus, the size of the trial will have to be such that, based on incidence of infection and follow-up rates, it will have the power to answer these questions.

CONCLUSION

The narrow diversity of Asian subtype E viruses, which is associated with a neutralization serotype, suggests that the subtype E virus is an optimal target for HIV vaccines. The subtype has maintained its predominance in Thailand for eight years and appears associated with much of the HIV epidemic throughout the rest of Southeast Asia, altogether more than a million infected people. An international consortium of industry, military and academic medicine is developing and testing the first HIV vaccines designed specifically for the subtype circulating in a developing region of the world. Determination of whether or not these HIV vaccines are protective can only be accomplished in a phase III clinical trial, the successful completion of which may require extensive regional collaboration and effort.

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