Transmission of HIV and Other Infections in Southeast Asia

Cultural and Genetic Factors

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INTRODUCTION

Southeast (SE) Asia is second only to Sub-Saharan Africa as the world’s most heavily HIV/AIDS affected region in terms of number of infections (UNAIDS, 2002). While epidemic spread of HIV-1 began relatively late in the region, largely in the 1988–1990 period, the virus has spread widely and rapidly, and affected many millions in this densely populated, ethnically variable and politically diverse region. SE Asia includes some of the world’s wealthier states (Singapore and Brunei), emerging economies (China, Thailand and Vietnam) and some of the poorest and most isolated nations (Burma and Laos). Its political spectrum spans emerging democracies, military dictatorships, Islamic Republics and three of the last five nominally communist states worldwide (China, Laos, and Vietnam). Also located in SE Asia are two of the world’s leading heroin exporters, Burma and Laos (Beyrer, et al., 2000). The ethnic and linguistic diversity of the region is extremely complex, as are the range of climates and ecologies—from rain forests and sparsely peopled mountains to overcrowded metropolises, with their associated socio-economic deprivations, poor housing and contaminated water supplies.

The range of infectious diseases affecting the peoples of SE Asia is as diverse as the peoples themselves: some parts of Burma and Laos are hyper-endemic zones for \textit{P. falciparum} malaria, much of the region is affected by seasonal dengue outbreaks, and a wide array of other infectious pathogens cause endemic or epidemic diseases including scrub typhus, filariasis, hepatitis A, B and C viruses, typhoid, cholera, rabies, schistosomes, helminths, and mycoses. Understanding the dynamics of HIV and other disease spread across the region, and the ways HIV-1 interacts with the underlying human and biologic complexity is a challenge to epidemiology, virology, human and viral genetics, as well as to the political and social
sciences. In a region as dynamic as SE Asia, attempting to understand these epidemics may yield vital insights into the future of HIV-1 spread, and the potential for vaccine induced control of this hyper-variable pathogen.

MAINLAND SE ASIA: LAND, POPULATIONS, LANGUAGES, ETHNICITY AND GENETICS

For the purposes of this exploration we can define mainland SE Asia as encompassing Burma, Thailand, Laos, Cambodia, Vietnam and Peninsular Malaysia; and three additional regions with close affinity to the mainland: Northeast India and Southeastern and Southwestern China. This area is often referred to as the Greater Mekong Subregion, for it is linked by the Mekong River and its tributaries (Figure 1). Geographic SE Asia is the tail end of the Himalayan mountain chain. It is cut by a series of great rivers, all of which come off the Tibetan Plateau to flow roughly South and East to the seas. The region can be seen as a series of these North-South running river valleys, which gradually fan out to highly fertile flood plains and deltas (Figure 1). These include the Irrawaddy, or Ayeyarwady, flood plain—the rice growing center of Burma; the Mekong, which supports the populations of Yunnan, Laos, Cambodia, and broadens to become the expansive Mekong Delta of South Vietnam; the Chao Phraya basin, the great central river system of Thailand; the Salween of Yunnan and the Shan States of Burma, and many other smaller bodies. These river valleys and lowland deltas have all been intensively cultivated for irrigated wet rice agriculture for millennia, and have long generated surpluses capable of supporting dense populations and advanced societies. The mountains above, however, have radically different climates and cultivation possibilities. Most of the chains are forested limestone Karsts, infertile and rugged. Because the valleys run north-south, each of the mountain ridges tends to be isolated from its neighbors, and little connection has ever been made by the mountain peoples across these ranges. While the dominant Burman, Thai, Lao, Vietnamese and Khmer rice growers of the lowlands built the great early civilizations of Pagan, Ayuthia and Angkor, the ethnically distinct and predominantly Sino-Tibetan mountain peoples still subsist on slash and burn agriculture and live in small shifting settlements little different from archaic times (Coedes, 1975). Only dry rice will grow in many of these mountain regions, and most are rainfall reliant. In the harshest zones, such as the stony Wa hill tracts of Burma, and the Karsts of northern Laos, the opium poppy, *Papaver somniferum*, has long been one of the few crops these tribal farmers could grow profitably (Beyer, 1998).

The overall population in mainland SE Asia is estimated to be 344 million persons. This number includes the populations of the mainland states, an estimated 100 million Chinese in Yunnan and Guangxi Provinces combined, and the roughly 10 million residents of the Northeast Indian States of Manipur, Mizoram, and Nagaland on the India-Burma borders. It does not include the population of the Philippines, whose HIV epidemic appears to be unlinked epidemiologically to mainland SE Asia (UNAIDS, 2002). The rational for the inclusion of the Indian and Chinese regions becomes clear if we look beyond modern political boundaries to the ethnic and historical relationships of these regions. For example, the Manipuri people, the predominant ethnic group in Manipur State on the India–Burma border, are close linguistic and ethnic cousins of the Burmese, and their language is in the same sub-group of Tibeto-Burman as modern Burmese. In like manner, the formal name for Guangxi Province in Southeastern China, is the Guangxi Zhaung People’s Autonomous Region. The Zhaung are a Daic people whose language is part of the same family which includes modern Thai, Lao, and Shan (the language of the Shan States of Eastern Burma) and which is unrelated to the Sino-Tibetan family to which modern
FIGURE 1. The Greater Mekong Subregion: Mainland Southeast Asia, Yunnan and Guangxi Provinces of China, and Northeast India.
Chinese belongs (Schliesinger, 2000). These linguistic relationships are recognized to be parallel markers of human genetic relationships (Cavalli-Sforza et al., 1988), and hence immunogenetic relationships which may play important roles in host components of infectious disease resistance and spread in the region.

The emerging field of human genetic history is yielding a wide range of insights into this complex area. It is increasingly clear that SE Asia was one of the first areas inhabited by ancestral populations after leaving Africa. Early inhabitants of mainland and insular SE Asia were in turn ancestral to the peoples of Australia, China, and Polynesia as far east as Hawaii. Remnant populations, such as the Andaman Islanders off the Burma coast and the Semang (a negrito pygmy tribal people) of southern Thailand, appear to be virtually direct descendants of some of the first modern humans to have left Africa (Schliesinger, 2000). While the earliest human population spread was most likely north into mainland Asia, the movement of peoples in historical times has nearly always been south, out of the huge and populous mainland of East Asia into peninsular SE Asia. The Burmans of Burma moved south from Tibet to conquer the ancient Mon peoples. The Thais (Daic speakers) came out of their homelands in what is now Yunnan Province, China to populate the major river valleys of the region such as the Mekong, the Chao Phraya and the upper reaches of the Red River in north Vietnam. This led to the domination by the Thais of the indigenous Lawa peoples of modern day Thailand, Shan State in Burma, and Laos. The Vietnamese from the north (the Red River Delta) eventually conquered the Chams of Central Vietnam and the Khmers of the Mekong River Delta in the South to unify Vietnam. Among the mainland SE Asian States, Cambodia, with her majority population of Mon-Khmer people, may be somewhat unique in being peopled predominately by an indigenous ethnic group.

**NARCOTICS AND HIV-1 DIVERSITY IN THE TRANSMISSION “HOT-ZONES”**

The confluence of southwest China, Burma, Thailand, and Laos is known as the Golden Triangle, and it has long been one of the world’s major opium poppy cultivation zones. As with other regions of high narcotic production, the insurgent wars between various ethnic groups and armed forces that have raged for the last 60 years in the Golden Triangle have exacerbated heroin production, trafficking, and its use in SE Asia (Linter, 1994). Given the centrality of injecting drug use to the early spread of HIV in SE Asia, it is clear that we cannot understand HIV-1 spread in the region without an examination of the role of heroin and injection drug use (Beyer, et al., 2000). China, India, Burma, Thailand, Malaysia, and Vietnam have all had severe HIV-1 epidemics among injecting drug users (IDU) (Crofts et al., 1998). In China, Malaysia and Vietnam, IDU account for the majority of reported infections; over 70% in all three countries. And across the region, IDU inject heroin, the refined product of opium poppy base. More than 90% of the region's heroin is from Burma, where the drug is exported as a highly pure injectable grade white powder called #4, for its four stage refinement (U.S. Department of State, 2003). In 2000, we reported on the relationship of overland trafficking of heroin from Burma and Laos on the HIV epidemics of the region (Beyer et al., 2000). Genetic analyses of HIV-1 sequence data from IDU along the regional heroin trafficking routes proved a powerful tool to understanding both HIV spread and the movement of narcotics and injection networks (Piyanrisilip et al., 2000; McCutchan et al., 2002; Takebe et al., 2003). For example, we would assume that since the major heroin route north out of Vietnam into China followed the principal road and train route from Hanoi to the border crossing point at Pingxiang City in Guangxi Province (Figure 2), the HIV-1 subtype found in Pingxiang IDU would
The similar to that found in HIV-infected IDU in northern Vietnam (Kato et al., 1999). This was indeed the case—both were highly homogeneous outbreaks of HIV-1 CRF01_A/E, generally referred to as subtype E (Yu et al., 1999). But this subtype did not spread widely after its introduction into Guangxi Province. This is not because there are not IDU populations inland from the Vietnamese border—IDU are found throughout the Province, and
indeed, another subtype of HIV-1 has spread widely among the drug users of central and northern Guangxi, a B/C recombinant virus that probably originated in upper Burma (Yu et al., 1999; Piyasirisilp et al., 2000; McCutchan et al., 2002; Takebe et al., 2003). A possible explanation may lie in the genetic make-up of the Guangxi Zhuang People’s Autonomous Region. Before exploring this relationship, we must consider some host-viral interactions which may be relevant to disease spread.

**HIV-1 MEETS HLA IN THE EAST**

Trade routes facilitating the distribution of “black” economy products such as narcotics, together with the high-risk practices of IDU and limited prevention programs, act respectively as networks of transmission and nodes of amplification of HIV-1, in the large and vulnerable populations of mainland SE Asia. However, the observed segregation of HIV-1 CRF01_A/E in the ethnic Thais, Khmer, and Vietnamese, and HIV-1 B/C recombinants in the Han remains a paradox, as these populations intermix and share high-risk activities in the transmission hot zones of northern Vietnam and south-west China, Burma proper, and almost certainly in the Burma-Yunnan border zones, where all the viruses discussed here have been found in varying relative frequency (Yu et al., 1999; McCutchan et al., 2002; Takebe et al., 2003). This segregation suggests a possible biological explanation for the population-based divergence of HIV-1 clades in SE Asians. Such a segregation could have an impact on future vaccine programs in the region, but could have wider impacts as well, as the relationship of population genetics to the spread of HIV-1 subtypes is little understood globally, and may become clearer, unfortunately, as HIV-1 continues to diversify as the pandemic reaches ever more human populations.

How might such population-pathogen interactions be mediated? Within the human genome, lies a dense cluster of genes on the short arm of chromosome 6, called the major histocompatibility complex or MHC, which spans some 3.6 megabases of DNA (Aguado et al., 1999). At least 51, or 40% of the expressed MHC genes encode proteins that are involved in both acquired and innate immune responses to microbes (Aguado et al., 1999). These include the classical class I and II human leukocyte antigens (HLA), which are transmembrane glycoproteins, whose biological function is to present endogenous (viral) and exogenous (bacterial) peptides to the antigen-specific T cell receptors of CD8+ cytolytic and CD4+ helper T cells, respectively. HLA class I molecules are also recognized by another group of receptors, that determine whether cells with innate natural killer (NK) activity spontaneously lyse virally infected cells (Parham, 2003). Thus, HLA class I molecules are essential recognition elements of both the acquired and innate immune responses to viruses.

The classical HLA class I (HLA-A, -B, -C) and class II (HLA-DR, DQ and DP) gene loci are highly polymorphic and each locus encodes hundreds of alleles, making these loci the most polymorphic loci in the human genome. The HLA-B locus is the most variable, encoding over 500 distinct alleles (see www.ebi.ac.uk/imgt/hla). This polymorphism is evident in different populations, as many alleles are detected at different frequencies, both within and between ethnic groups, be they African, Asian or Caucasian in origin. Another feature of HLA class I and II genes is the phenomenon of linkage disequilibrium, where certain combinations of alleles encoded by different loci, tend to form stable haplotypes, which are inherited from one generation to another in a Mendelian fashion. Again, the composition and frequency of HLA haplotypes also varies between different ethnic groups. Thus, HLA class I and II alleles are recognized to be excellent markers of ethnicity, and the analysis of these genes has proved to be highly informative in anthropological studies (Cavalli-Sforza et al., 1988).
However, the extreme polymorphism of HLA class I and II genes has a huge influence on the biological function of their products. Most of the polymorphism of these genes results in significant amino-acid changes in the first and second extracellular protein domains of HLA molecules, which affect the binding and subsequent presentation of microbial peptides to antigen-specific T cells. This has led to the concept that there are groups of HLA class I alleles or supertypes, with similar preferences for short antigenic peptides or supermotifs that share a similar structure (Sidney et al., 1996). Our current understanding of HLA class I and II binding preferences, in terms of the size and composition of their bound peptides, contributes to the rational selection of target antigens in modern vaccines.

Fortunately, considerable information is available on the phenotypic and genetic diversity of HLA class I and II alleles and haplotypes in mainland SE Asian populations (Chandanayyingong et al., 1994 and 1997; Stephens et al., 2000). This is largely because high quality HLA typing was set up in Bangkok, Thailand, in the early 1970s and such work has further developed since. Ethnic Thais residing in Thailand and the related Dai, Dai Lue, Shan, Lao Loum, Black Tai, White Tai, and Red Tai ethnic groups in Burma, Yunnan and Guangxi Provinces of Southwest China, Laos, Cambodia, and Vietnam, comprise some 70 million people sharing a common language, spoken dialects, religion (Theravada Buddhism), architecture, agricultural practices, and culture (Chandanayyingong et al., 1994). Ethnic Thais in Thailand proper are relatively homogeneous, probably because Thailand, unlike its neighboring countries, avoided European colonization in the 19th century. There is clear evidence from the analysis of HLA allele and haplotype profiles that Thai populations are highly representative of the genetic pool present in the large populations of this region (Chandanayyingong et al., 1994 and 1997; Stephens et al., 2000). For example, certain HLA-DR2 related class II allele and haplotype profiles that are characteristic of Thais (Stephens et al., 2000), are also detected at similar frequencies in the dominant Vietnamese Kinh population of Vietnam (Vu-Trieu et al., 1997), the Khmers of Cambodia (Goldfield et al., 1998) and Indonesians from Java (Gao et al., 1991). This is entirely consistent with the ethno-linguistic similarities and historic migrations between populations of the SE Asian mainland and the Indonesian archipelago (Stephens et al., 2000). However, the same common DR2-related haplotypes, such as DRB1*1502, DRB5*0101, DQA1*0102, DQB1*0501, are rare in the dominant Han Chinese (non-Thai) populations of SW China (Stephens et al., 2000, Gao et al., 1991). Thus Thai-like genetic profiles dominate in much of SE Asia, and Han profiles dominate in China proper. The “mixing zone” of these two ethno-linguistic branches is the southern China border regions with Burma, Laos, and Vietnam, which corresponds to the Chinese Provinces of Yunnan and Guangxi, precisely where the two major recombinant forms of HIV-1, CRF01_A/E and the group of B/C recombinants, are in circulation.

There is also evidence for inverse north-south gradients or clines of HLA allele and haplotype frequencies across the populations of SE Asia (Chandanayyingong et al., 1994 and 1997; Stephens et al., 2000). For example, the HLA-B22 group of class I alleles (HLA-B54, B55, B56), which have been associated with fast progression of HIV/AIDS in Caucasoids (Hendel et al., 1999), is far more common in the Han groups of SW China than in ethnic Thais (Chandanayyingong et al., 1997). Conversely, other major HLA class I and II alleles such as HLA-A33, B44 and DR7, which together can form a stable haplotype, are clearly more frequent in Thais and Vietnamese than in the southern Han of SW China (Chandanayyingong et al., 1997). Herein lies a possible explanation for the apparent divergence of HIV-1 B/C variants into the more northerly Han populations of SW China and Burma, in contrast to
HIV-1 CRF01_A/E disseminating through the more southerly Thais and their HLA compatible Vietnamese Kinh and Cambodian Khmer populations. Namely, the current separation of HIV-1 clades in the large and diverse populations of SE Asia appears to correlate with major divisions within the immunogenetic profiles of those populations currently at risk of HIV-1 exposure.

THE HLA-A11 CONNECTION IN THAILAND

Detailed analyses of HIV-1 seroconverters have clearly shown that the presence or absence of certain HLA class I alleles correlates with the rate of developing AIDS in North American Caucasoids and African-Americans (Carrington et al., 1999; Gao et al., 2001). A similar picture has emerged from studies performed on HIV-1 infected cohorts identified in Africa (MacDonald et al., 2000) and Western Europe (Hendel et al., 1999; Flores-Villanueva et al., 2003). There is also evidence that genes encoding certain HLA class I alleles and NK receptors are acting together to influence survival in the North American HIV-1 seroconverters (Martin et al., 2002). By contrast, very little information is available on the influence of HLA gene and allele profiles on the development of AIDS in Asian HIV-1 seroconverters. However, a variety of studies involving cohorts of HIV-1 exposed ethnic Thais (Beyrer et al., 1999; Sriwanathana et al., 2001) are worth further review in the context of what may be the genetic basis of protective immune responses to HIV-1 infection in mainland SE Asians.

At the Berlin International AIDS conference of 1993, the first reports were revealed of HLA class I alleles associating with an apparent resistance to HIV-1 infection in a group of East African female sex workers (FSW) (Plummer et al., 1993). These women had been highly exposed to HIV-1 but remained persistently seronegative (HEPS). Soon after, HLA class I restricted CTL responses to a variety of immunogenic peptides derived from HIV-1 Gag, Pol and Nef proteins, were also described in a similar cohort of West African HEPS FSW (Rowland-Jones et al., 1995). Around the same period, a small but highly informative cohort of ethnic Thai HEPS FSW were identified in Chiang Mai and Chiang Rai in Northern Thailand (Beyrer et al., 1999; Sriwanathana et al., 2001), a region where the first introduction of HIV-1 clade CRF01_A/E had occurred some 10 years previously. It quickly became clear through HLA typing of these Northern Thai HEPS FSW that a significant proportion of these women were carrying the HLA-A11 antigen, as identified by classical serotyping in Bangkok (Beyrer et al., 1999; Sriwanathana et al., 2001). Molecular confirmation of alleles encoding HLA-A11 antigens, using a variety of PCR-based techniques including direct sequencing, has demonstrated a preponderance of HLA-A*1101 in these HEPS FSW (Stephens and Chandanayangyong, unpublished observations). Given that HLA-A11 is a prevalent class I antigen in SE Asian populations (Chandanayangyong et al., 1997), the presence of the A*1101 allele in the Northern Thai HEPS FSW came as no surprise, at least to the HLA researchers in Bangkok. However, the frequency was substantially higher when compared to the known Northern Thai population frequency for HLA-A11 (Beyrer et al., 1999; Sriwanathana et al., 2001). This suggested that a common host cytolytic mechanism, directed against HIV-1 and utilizing the HLA-A11 molecule, was occurring in the Northern Thai HEPS FSW.

HIV-1 NEF—A SUITABLE CANDIDATE FOR PRESENTATION BY HLA-A11 IN ETHNIC THAIS

By the mid-1990s, the Los Almos database had already accrued considerable
information on HIV-1 immunogenic peptides, known to drive HLA class I allele-restricted antigen-specific CTL and proliferative responses in different populations (Korber et al., 1996). From this database, it was reasonably easy for researchers in Bangkok to predict which potential peptides were likely to be driving HLA-A11 restricted CTL responses in ethnic Thais. The HIV-1 Nef protein came under close scrutiny, as this product was known to down-regulate HLA expression on HIV-1 infected cells, a molecular mechanism used by a variety of viruses to evade immune recognition, and it also acted as early infectivity factor for the virus (Collins et al., 1998). One particular HIV-1 Nef-derived peptide, QVPLRPMTYK (positions 73–82), came under particular attention in Bangkok. This peptide had already been used to demonstrate HLA-A11 restricted CTL activity against HIV-1 in asymptomatic HIV-negative partners in discordant Caucasoid couples (Langlade-Demoyen et al., 1994) and in asymptomatic HIV-positive Caucasoids (Coullin et al., 1994), as well as CTL activity in West African HEPS FSW (Rowland-Jones et al., 1995). In turn, the same HIV-1 Nef positions 73–82 peptide also proved to be a target of HLA-A11-restricted CTL activity in some of the Northern Thai HEPS FSW (Sriwanathana et al., 2001).

It was also apparent, by the mid-1990s, that HIV-1 Nef pos 73–82 was under direct immunological pressure. Sequencing of HIV-1 clade E isolates from infected Thais had shown a variety of mutations in this peptide (Artenstein et al., 1996), which had probably occurred within the 10 years since the introduction of HIV-1 clade CRF01_A/E into this population. However, the mutations do not appear to affect positions 74 and 82 of the HIV-1 Nef 73–82 peptide (Artenstein et al., 1996), which are thought to be the critical anchor positions of the HLA-A11 binding antigen supermotif, recognized by the HLA-A3 supertype of HLA class I alleles (HLA-A*0301, A*1101, A*3101. A*3301 and A*6801), which share common preferences in the types of peptides they bind (Sidney et al., 1996). Indeed, the putative HLA-A11 supermotif anchor residues Val74 and Lys82 present in Nef 73–82 peptide appear to be highly conserved in all clades of HIV-1, as well as HIV-2, SIVmac and SIVcpz (Korber et al., 2002), suggesting that they are biologically necessary for productive infection. Furthermore, the region of HIV-1 nef containing the 73–82 peptide is saturated with overlapping CTL epitopes, recognized by a variety of class I alleles, such as HLA-A2, -A3, -A11, -B7, -B8, -B27, -B35 and -B60 (Korber et al., 2002), which are common in many populations, including SE Asians (Chandanayingyong et al., 1997). Taken together, this CTL epitope-dense region of the HIV-1 Nef protein, may be a strong contender for inclusion in future vaccines designed to reduce viraemia and induce protection against HIV-1 exposure.

**HLA-A11—A SUITABLE CANDIDATE FOR NK RECOGNITION**

Our current understanding of mature, circulating, cytolytic T cells is that they express a variety of receptors that mediate the lysis of virally infected cells (Ugolini and Vivier, 2001). Many of these receptors facilitate innate NK lysis of target cells. There are two major groups of NK receptors, the killer immunoglobulin-like receptors (KIR) and the lectin like receptors (NKG2), encoded on chromosomes 12 and 19, respectively (Middleton et al., 2002). Depending on the structure of their cytoplasmic domains, KIRs can either functionally activate or inhibit NK lysis after interacting with their ligands, which are HLA class I molecules (Middleton et al., 2002). Some NK KIR gene loci are ubiquitous and present in all individuals, while other loci are polygenic and can be
either present or absent in certain individuals (Norman et al., 2001 and 2002). NK KIR gene loci are also highly polymorphic (Carrington and Norman, 2003; Marsh et al., 2003), and at least one locus (3DS1) has been associated with AIDS survival (Martin et al., 2002).

One of the ubiquitous NK KIR gene loci, called 3DL2, encodes an inhibitory receptor that only recognizes the HLA-A3 supertype of class I alleles, including HLA-A11 (Pende et al., 1996; Middleton et al., 2002). Under normal circumstances, when 3DL2 interacts with HLA-A11, an inhibitory signal is transmitted to the NK cell, and lysis of the target cell is prevented. However, there is evidence that when HLA-A11 molecules are loaded with viral peptides in vitro, inhibitory signaling is curtailed and NK lysis of targets resumes (Gavioli et al., 1996). The KIR-3DL2 locus has been detected in all individuals tested so far, including ethnic Thais (Norman et al., 2001 and 2002). KIR-3DL2 would also appear to be the most polymorphic of the KIR receptors (Marsh et al., 2003) and it forms dimers on the surface of NK cells (Pende et al., 1996). Thus, we have a highly polymorphic NK receptor, probably expressed as a dimer in all ethnic Thais, which specifically recognizes HLA-A11 that can also dimerize (Li et al., 2002). The overall outcome of this interaction, in terms of NK lysis, may depend on whether HLA-A11 is loaded with viral peptides. Could this be pure coincidence with the prevalence of HLA-A11 in the Northern Thai HEPS FSW? Or is it a serendipitous correlation with resistance to HIV-1 infection? Such issues may be resolved by further analysis of NK receptor gene and allele profiles in this unique cohort of HIV-1 exposed but uninfected mainland SE Asians. At present, the biological relevance of NK KIR polymorphism is unclear, but there are certainly allelic variations between different ethnic groups (Norman et al., 2001 and 2002), which, in turn, may correlate with the known population variations of NK activity in HIV-1 infected individuals, including ethnic Thais (De Souza et al., 2000).

**RELEVANCE TO OTHER ENDEMIC INFECTIONS—TUBERCULOSIS, MALARIA, DENGUE, HEPATITIS B AND C**

**Tuberculosis**

The analysis of HLA allele associations with infectious diseases in SE Asian populations remained a relatively neglected field until the early 1990’s. However, considerable evidence has since accumulated suggesting that HLA alleles associate with the severity of dengue virus infections, (Stephens et al., 2002), typhoid fever (Dunstan et al., 2001), hepatitis C (Veijbaeya et al., 2000), melioidosis (Darakul et al., 1998), malaria (Stephens et al., 1995; Hirayama et al., 1996; Buson et al., 2002), and tuberculosis (Goldfield et al., 1998; Veijbaeya et al., 2002), which are all endemic in mainland SE Asia.

Much concern has developed over the inevitable occurrence of co-infections of HIV-1 with malaria or tuberculosis. Throughout the region, tuberculosis is the most common opportunistic infection seen in clinical AIDS, and TB rates among young adults have expanded dramatically in Thailand, Burma, and Cambodia (Charirialertskak et al., 2001). Re-activation of previous exposure to *Mycobacterium tuberculosis* is well documented in HIV-1 infected Northern Thais (Yanai et al., 1996). Worldwide there is considerable evidence that HLA-DR2 related alleles and haplotypes, which are very common and diverse in SE Asians (Gao et al., 1991; Chan-danayingyong et al., 1997; Stephens et al., 2000), also associate with susceptibility to developing tuberculosis (Khomenko et al., 1990). However, immunogenetic studies testing for associations between HLA alleles and the development of latent TB infections in
HIV-1 exposed individuals have yet to be performed.

Malaria

Malaria is an important pathogen with a strikingly uneven distribution in the region. Malaria morbidity and mortality rates are highly variable across the region, and correlate with the vector's preferred habitat of mountain forests and their resident ethnic groups. Malaria death rates are low in Southern China, most of Thailand, and most of Vietnam (Kidson et al., 1999). By contrast, malaria disease and death rates are very high, in the 50–150/100,000 population range, in forest and mountain parts of Burma, most of Laos, and some parts of Cambodia (Nihei et al., 2002). Labor-intensive forest encroachment for the logging of hard woods and mining of natural resources has increased the risk of exposure to malaria and co-infection with HIV-1, as co-infection among Burmese migrant workers in these zones is not uncommon. Chaotic and under-funded essential drug programs in Burma, Laos and Cambodia appear to have contributed to multi-drug resistant *P. falciparum* malaria outbreaks, and *P. falciparum* strains from these states are commonly resistant to chloroquine, antifolates, quinine, and mefloquine. (Wongsrichanalai et al., 2002). Resistance to artesunate and artemisinin has also been found among *P. falciparum* isolates from the region (Picard et al., 2003). For Thailand, malaria is virtually synonymous with border areas: her cases come largely from the forest/mountain regions on her western border with Burma, and her eastern border with Cambodia (Kidson, 1999). The ongoing civil conflict in Burma contributes substantially to the malaria disease burden: urban Burmese continue to flee east to Thailand through the dense malaria zones of the Thai–Burma border, many encountering forest malaria for the first time in adult life. Much of the malaria morbidity and mortality in this region occurs among Burmese migrants and refugees (Chareonviriyaphap et al., 2001). While malaria disease rates are highest in Burma, Laos and Cambodia, the mortality differences also likely reflect much more limited health services and hence, death rates from cerebral malaria, renal failure, and the like. Clearly, this will be true for AIDS mortality as it is for malaria deaths: Thai citizens already have some access to anti-virals, while Burmese and Laotians, particularly in the mountain zones, cannot access HIV testing, much less AIDS care (Cohen, 2003). While Laos remains a very low prevalence country for HIV-1, Burma has among the most severe epidemics in the region, with a population prevalence estimated at 3.46% of reproductive age adults (Beyrer et al., 2003b). Cambodia has the second highest HIV rate in the region, and high malaria morbidity and mortality as well. HIV-1 and malaria interactions are, therefore, likely to be problematic, mainly in Burma and Cambodia: both countries with multi-drug resistant *P. falciparum*.

Dengue Virus

Dengue virus infections in SE Asia appear to follow a very different pattern from malaria or HIV-1. The primary vector for this acute virus infection is the *Aedes aegypti* mosquito. This mosquito thrives in both the sprawling tropical capitals of the region and in rural environments. Dengue virus attack rates do not track disturbances in civil society or heroin trafficking routes through mountain ranges. However, outbreaks of dengue have been increasing in frequency and severity in the region. This phenomenon is little understood, but seems to correlate with temperature fluctuations associated with recent climatic changes, such as the “El Nino” phenomenon. Primary and secondary infections can occur with all four of the major dengue virus serotypes, which are in seasonal circulation in SE Asia. Exposure to dengue virus
can result in either asymptomatic infections, clinical dengue fever (DF), or the more severe dengue hemorrhagic fever (DHF). Both DF and DHF are essentially untreatable except for supportive therapy in the case of DHF. In secondary dengue virus exposures, a memory immune response is induced that can both clear the infecting virus and contribute to disease pathology. There is clear evidence in ethnic Thais that certain HLA class I alleles associate with clinical outcome of secondary dengue exposure and the infecting virus serotype in previously exposed and immunologically primed individuals (Stephens et al., 2002). Furthermore, the analysis of HLA-A11 restricted responses to dengue during the acute viraemic phase of infection in ethnic Thais has indicated that some CTL responses may be stunned or ineffective in secondary infections (Mongkolsapaya et al., 2003). However, HLA-A11 would only appear to associate with susceptibility to severe secondary infections with one particular dengue serotype (Stephens et al., 2002), and a detailed analysis of CTL responses driven by HLA class I alleles that associate with resistance to severe disease has yet to be performed. Nevertheless, the possibility that stunned cross-reactive CTL responses occur after secondary or multiple exposures to different clades of HIV-1 in SE Asians remains unexplored, although this possibility may have an impact on the efficacy of future HIV-1 vaccines in this region.

**Hepatitis B and C Virus**

East Asian populations have long been known to have high endemic rates of Hepatitis B virus (Nelson and Thomas, 2001). Indeed, field trials of HBV vaccines were conducted in Taiwan in the 1980s due to the exceptionally high population prevalence among reproductive age adults there (Beasley et al., 1981). These HBV rates appear to play a role in making hepatocellular carcinoma one of the leading causes of cancer death in China and SE Asia. HCV in these populations is distinctly different. While population rates of HBV have long been high, HCV has seen explosive spread through injecting drug use, and hence has moved along the same heroin-IDU pathway in the region as HIV-1. HCV rates in IDU are very high in the region wherever they have been assessed. Indeed, in cohort studies among drug users in Chiang Mai, rates were so high (over 90%), that an analysis comparing HCV positive and negative IDU was impossible due to a classic "ceiling effect" (Razak et al., 2003). Similar rates have been found in Chinese populations, though many fewer studies, relative to the scale of the epidemic, have been done. Preliminary disease association studies of HCV in ethnic Thais have indicated that certain HLA class II alleles associate with both transient and persistent HCV infection, as well as overall protection against disease (Vejbaeysa et al., 2000). Since HCV, like HIV, has multiple subtypes, it may be that HLA and ethno-linguistic associations with this virus would help elucidate HCV epidemiology in the region.

**PUBLIC HEALTH AND THE PROSPECTS FOR HIV PREVENTION AND CONTROL**

Whatever the genetic relationships between HIV variants and human populations, two things are abundantly clear: preventive measures can work, and they are currently underused. HIV-1 epidemics among drug users have been contained and reduced in a number of countries including Australia, Brazil, the Netherlands and the UK (Des Jarlais and Friedman, 1998). Provision of those tools known to have efficacy for prevention of IDU transmission, including harm reduction, needle and syringe exchange, and drug treatment (methadone and buprenorphine on demand) can have major impacts on reducing the spread of blood-borne pathogens among injectors and their sex partners (Des Jarlais and Friedman, 1998). When we look across SE Asia, China, and India, however, the use of these
tools is limited or absent (Crofts et al., 1998). Among Asian nations, only Hong Kong provides both harm reduction and modern drug treatment on demand.

If prevention services are lacking for drug users in Asian communities, they are even more severely limited in Asia's prison systems. While very little information is available from many such prisons, it is clear in the case of the Thai epidemic that the Bangkok prison system played a key role in the early spread of HIV in the country (Choopanya et al., 1991). In the Thai prison system, drug treatment is limited, needles and syringes are unavailable, but heroin is available, at least in some settings. This means that needle and equipment sharing is the norm for incarcerated drug users, and time in prison has been shown to be causally linked to HIV seroconversion among incarcerated drug users (Choopanya et al., 2002). In the Choopanya study, the HIV infection rate among incarcerated drug users was over 30% per year, as opposed to less than 4% per year among those out of prison. Such high transmission prison settings may both concentrate and mix IDU from diverse networks and regions, and may play a crucial, but little understood, role in the dissemination of HIV subtypes.

Where sexual spread predominates, in Cambodia, Thailand, and parts of Burma, prevention has been strikingly more successful for those at sexual risk than among IDU. Both Thailand and Cambodia have seen substantial declines in HIV infection rates among pregnant women, indicating a general decline in new infections among sexually active adults (UNAIDS, 2002). Early on, Vietnam embraced and adapted the Thai prevention model (the 100% Condom Campaign for commercial sex venues) and may have largely prevented heterosexual spread. Unfortunately, however, the limitations on prevention for IDU means that ongoing sexual spread from IDU to their wives and girlfriends will continue in most SE Asian states. This may allow for further study of the host–viral pathogen interactions we have discussed.

In many respects, the immunological stage is now well set in terms of the HIV-1 epidemic in mainland SE Asian populations. Concurrent epidemics of HIV-1 CRF01_A/E and B/C variants are moving rapidly through some of the most densely populated regions of the world. The size and homogeneity of SE Asian populations contrast starkly with the relatively lower density but extreme genetic diversity of African populations at risk of HIV-1 exposure. There is already good evidence that the HIV-1 viruses, emerging after circulating for many years in Caucasoid HIV-1 seroconverters, are assiduously mutating in the key immunogenic regions or epitopes that are recognized by the prevalent HLA alleles in these populations (Moore et al., 2002). If HLA is molding the HIV-1 virus in Caucasoids, then it is reasonable to assume that a similar phenomenon is occurring in SE Asians, as indicated by sequencing studies of viruses derived from infected ethnic Thais (Artenstein et al., 1996), the original seed population of the SE Asian epidemic. The extensive information already available on immune response (HLA) gene and allele profiles in SE Asians will enable and facilitate further large-scale population-based investigations, with the power to make meaningful regional extrapolations on the genetic basis of susceptibility and resistance to HIV/AIDS. This can be achieved by utilizing local expertise and knowledge through establishing regional networks of collaborations, which could contribute to the rational design of future vaccines.

SE Asia will likely remain a region of sharp contrasts. While the modern Thai state is an emerging democracy and a regional economic power, Laos remains on the United Nations list of the world's 10 least developed nations. Malaria deaths are tragically common along the Thai–Burma border, while almost unheard-of in urban Bangkok. The regional narcotics economy shows no sign of abating, and HIV-1 spread continues apace in Southern China, threatening an enormous population. While these are challenges
for individuals, communities, and countries, they are also regional challenges, and are likely to require regional solutions. This is well understood by public health authorities in the region, and increasingly by international donors. HIV/AIDS and malaria efforts are increasingly managed under a Greater Mekong Subregion framework within WHO and UNDP, for example. And the regional political body, ASEAN (The Association of Southeast Asian Nations) has public health task forces working on HIV/AIDS, tropical diseases, and increasing partnership across political borders. Such collaborations may be the key to addressing these regional challenges to the health of the people of SE Asia. It would not be the first time that public health has led the way in regional cooperation and improved relations. The peoples of the region certainly deserve no less.

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