Published in final edited form as:
J Infect Dis. 2009 May 15; 199(10): 1442–1448. doi:10.1086/597422.

Tumor necrosis factor (TNF) and lymphotoxin-alpha (LTA) gene, allele, and extended HLA haplotype associations with severe dengue virus infection in ethnic Thais

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Abstract

Severe dengue virus (DENV) infections are characterized by a cascade of cytokine production including TNF and LTA. We have analyzed a variety of polymorphisms in the TNF and LTA genes of 435 ethnic Thais with subclinical DENV infection, primary and secondary dengue fever (DF), and dengue hemorrhagic fever (DHF). The TNF -238A polymorphism marking the TNF-4, LTA-3 haplotype, was significantly increased in patients with secondary DHF (15.2%) compared to secondary DF (4.1%) (P= 0.0009, Pc=0.022; OR = 4.13, 1.59<OR<11.17). In a subset of patients the LTA-3 haplotype associated with *in vivo* intra-cellular production of LTA and TNF during the acute viraemic phase of infection. Two extended MHC haplotypes containing TNF-4 and LTA-3, together with HLA-B48, B57 and DPB1*0501, were detected only in patients with secondary DHF. These observations indicate that polymorphism in functionally distinct MHC-encoded proteins contributes to the risk of developing severe secondary DENV infection and warrants further investigation.

Keywords

Dengue; Disease Severity; Genetic Associations; TNF; LTA; HLA; Ethnic Thais

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The authors declare no commercial or any other association that might pose a conflict of interest in this study.

This study was presented in part at plenary sessions of the British Society of Histocompatibility and Immunogenetics Annual Conference held at Warwick University (UK) on September 12th 2007, and the Second International Conference on Dengue Fever and Dengue Hemorrhagic Fever held in Phuket Thailand on October 16th 2008.

Introduction

Dengue virus (DENV) infections are an important cause of morbidity and mortality in tropical and subtropical regions including mainland SE Asia, with estimates of up to 100 million worldwide infections occurring yearly [1]. Human infections can be subclinical, or manifest as a self-limited febrile dengue fever (DF) or the more severe life-threatening dengue hemorrhagic fever (DHF) [2]. Illness can occur during either primary or secondary infections, although most patients who develop DHF are undergoing secondary infection with a virus serotype heterologous to their previous exposures [3].

DHF is characterized by increased capillary permeability resulting in leakage of plasma, potentially culminating in hemorrhage, circulatory shock and death. It has been proposed that DHF in secondary DENV infection is the immunopathological consequence of overproduction of various cytokines or chemical mediators [4]. Previous studies have shown increased serum and plasma levels of cytokines including tumor necrosis factor alpha (TNF) and lymphotoxin-alpha (LTA) in patients with DENV infections [5-8]. Relatively higher levels of TNF and LTA have been detected in ethnic Thais with DHF than DF, with peak levels occurring around the period of crisis when plasma leakage, hemorrhage, and circulatory shock commonly occur in DHF [7,8].

TNF and LTA are pleuripotent vasoactive immunomodulators produced mainly by activated monocytes and lymphocytes, respectively. TNF and LTA share the same receptors [9], many biological activities, and are central mediators of the immune response [10]. Both TNF and LTA are encoded by adjacent gene loci in the central or class III region of the human major histocompatibility complex (MHC), in between the human leukocyte antigen (HLA) class I and II genes on the short-arm of chromosome 6 [11]. TNF and LTA share some 50% homology in amino-acid composition. As with all eukaryotic genes, TNF and LTA expression is controlled by a series of cis-encoded nucleotide promoter motifs and trans-acting protein factors [12-14]. A variety of single nucleotide polymorphisms (SNP) have been identified in non-coding promoter-like regions, adjacent to exons encoding TNF and LTA [15]. Furthermore, linkage disequilibrium between TNF, LTA, and other HLA class I and II genes within the MHC contributes to the formation of haplotypes or stable combinations of SNP-defined alleles, that vary in composition and frequency both within and between different ethnic groups [15-17].

Case-control studies in a variety of populations have revealed associations between putative TNF promoter polymorphisms and malaria [12,18], leishmaniasis [19], leprosy [20], typhoid fever [21], and hepatitis B and C [22,23]. To date, the analysis of TNF gene associations with DENV infection have been limited. A case-control study of Vietnamese DHF patients identified no significant associations [24]. By contrast, another smaller study conducted in Venezuelans revealed an increased frequency of a putative TNF promoter polymorphism in patients with DHF [25]. The purpose of our current study was to examine SNP-defined TNF and LTA haplotype profiles in a cohort of ethnic Thais with either subclinical DENV exposure or primary and secondary DF and DHF.

Materials and Methods

Patients and controls

Unrelated ethnic Thai children with serologically and virologically confirmed DENV infections between the ages of 3 and 14 years and weighing > 20 Kg were enrolled in two clinical studies as previously described [26]. Clinical diagnoses of DF and DHF were assigned by an expert physician reviewer guided by World Health Organisation (WHO) criteria [2]. Two hundred and twenty-eight patients were diagnosed with DF and 142 patients were

diagnosed with DHF. DHF was further classified by severity according to the WHO criteria [2]. Serological responses to DENV were measured by both enzyme immunoassay and hemagglutination inhibition test. Primary or secondary infection was defined on the basis of the ratio of DENV-specific IgM:IgG serum antibodies and the titer of hemagglutination inhibition antibodies, according to WHO guidelines [2]. DENV serotypes (DENV-1, -2, -3 and -4) were identified by isolation in *Toxorhynchites splendens* mosquitoes and RT-PCR, as previously described [27-29]. 65 children were identified as having sub-clinical DENV infection, as indicated by increases in DENV-specific antibody titers without recognized illness, as previously described [29]. One hundred and forty-three unrelated ethnically-matched normal healthy Thais were used as controls.

Analysis of TNF and LTA polymorphisms

Full-length genomic DNA was isolated as previously described [30]. The polymerase chain reaction (PCR) sequence-specific primer (SSP) or phototyping method of Fanning et al [15], was used to identify TNF polymorphisms at positions -308 (G/A), -238 (G/A) and +488 (G/A), and LTA polymorphisms at positions +249 (A/G), +365 (G/C), and +720(C/A). This method employs 24 primer pairs to define cis-encoded polymorphisms at the above positions, with the following underlined modifications made to primers 708 (5'-ATAGGTTTTGAGGGGCATGG-3') and 709 (5'-ATAGGTTTTGAGGGGCATGA-3'). PCR reaction products were separated with electrophoresis (20 min at 200 V/cm^2) in 1 % agarose gel containing ethidium bromide and digital images of each gel recorded under UV illumination. The presence of a polymorphism-specific product of the expected size [15], in conjunction with an internal PCR control product, was considered to be positive. The frequencies of cis-encoded TNF and LTA polymorphisms defining the haplotypes TNF-1,-2,-3, -4 and LTA-1,-2,-3 [15], were determined using the following formula: haplotype frequency or HF (%) = n/N × 100, where n = the number of individuals scoring positive for the given haplotype and N = the total number of individuals genotyped in the patient or control groups. All of the DENV-exposed subclinical and clinical cases (n=435) and controls (n=143) were TNF genotyped. In addition, 361 clinical cases in the dengue group were LTA genotyped, as were all the controls. HLA class I and II allele profiles were also determined in selected patients and controls using both serological and molecular methods, and were defined using the standard WHO-recognized system of nomenclature for HLA serotypes and genotypes [26,30,34].

Immunocytochemical staining of TNF and LTA

Seventeen of the patients in this study had previously been included in a study of cytokine producing cells in peripheral blood [6]. A subset of patients with DF and DHF were selected at random, and TNF and LTA-specific monoclonal antibodies were used to determine the presence and frequency of cytokine-producing mononuclear cells [8]. Briefly, blood specimens were drawn daily from DENV infected patients during the febrile period, after defervescence, and at the early convalescence phase 5-8 days after defervesescence. Peripheral blood mononuclear cells (PBMC) were cryopreserved and stored in liquid nitrogen. Cells were thawed and cytospin preparations produced prior to the identification and enumeration of TNF- and LTA-producing cells using immunocytochemical staining and light microscopy [8]. The range of cells scoring positive for TNF production was 3-219 per 10^6 PBMC, and 3-27 per 10^6 PBMC for LTA production, with peak scores occurring mainly during the immediate defervescence period.

Statistical analysis

Hardy-Weinberg equilibrium tests were performed on all TNF and LTA SNPs in all patient and control groups as previously described [35], and all SNPs were found to be in equilibrium

except the TNF-308 in the secondary DHF group (data not shown). A variety of TNF and LTA HFs were compared between patient and controls using the Chi-square test for heterogeneity (2 × 2 contingency tables), with Yate's correction applied when necessary and Fisher's exact test used when the sum of adjacent fields was <10. P values < 0.05 were considered significant and corrected (Pc) for random haplotype associations (Bonferroni's) depending on the number of comparisons made, which included the number of TNF and LTA haplotypes identified (n=4 and 3 respectively), the number of patients and control groups (n=6), and the number of TNF and LTA haplotype combinations (n=12) or profiles (n=24) detected. Pc values < 0.05 were considered highly significant. The odds ratio (OR) with 95% confidence interval were used to assess the risk of disease severity associated with specific TNF or LTA haplotypes. *A posteriori* power calculations were also performed on all patient and control groups, using the PS (Power and Sample Size) and PGA (Power for Genetic Association Analyses) software programs [36].

Results

TNF and LTA haplotypes in dengue patients and controls

Using the PCR-SSP typing method of Fanning et al [15], which employs a series of short-range amplifications to unambiguously identify cis-encoded polymorphisms, we were able to determine the frequency of four combinations or haplotypes of TNF polymorphisms in all our patient and control groups (Table 1). The TNF-3 haplotype, which is characterized by a G to A substitution at position +488, was significantly decreased in frequency in primary DF, when compared to sub-clinical infections, patients with secondary DF or the normal healthy controls (Table 1). However, the most significant association we observed was with the TNF-4 haplotype, which is characterized by a unique G to A substitution at position −238, and was substantially increased in patients with secondary DHF compared to patients with secondary DF or normal healthy controls (Pc < 0.05) (Table 1). The TNF-4 association was also evident when all patients with both primary and secondary DHF were compared to the combined DF (Chi Square = 8.7, P=0.003, Pc =0.048; OR = 2.9) or control groups (Chi Square = 4.5, P=0.04, Pc =n.s.; OR =2.3), but of relatively lower significance than in patients with secondary infections alone (Table 1), indicating that the major association with this haplotype is with patients with secondary DHF.

By contrast, our analysis of LTA polymorphism using the method of Fanning et al [15], revealed the presence of 3 LTA haplotypes but no associations with any of the dengue patient groups (Table 2). Nevertheless, linkage disequilibrium between TNF-4 and LTA-3 haplotypes was evident. All but one of the patients and controls with TNF-4 that were LTA typed also carried the LTA-3 haplotype. Thus, the frequency of TNF-4, LTA-3 positive individuals in the secondary DHF patients (17/129 or 13.2%) was significantly higher than in patients with secondary DF (6/163 or 3.7%) (Chi square = 9.5, P = 0.002, Pc = 0.024, OR = 4.1 [1.5<OR<12.1]). We also stratified our data for the known infecting viral serotypes (DENV-1, -2, -3 or −4), but found no associations with any TNF or LTA haplotypes (data not shown).

LTA haplotypes correlate with in vivo LTA and TNF production

Seventeen patients that were TNF and LTA genotyped (DF n=9, DHF n=8), had previously been assessed for intracellular production of TNF and LTA during the acute viraemic phase of infection [8]. We therefore compared TNF and LTA haplotype profiles with the known presence or absence of cytokine production in these patients. No significant associations were detected between intracellular cytokine production and any TNF haplotype (data not shown). However, the LTA-3 haplotype associated with detectable LTA and TNF production (P<0.05, Table 3). By contrast, the LTA-2 haplotype correlated with a lack of TNF (P<0.05, Table 3) but not LTA production (data not shown).

Long-range extended TNF, LTA, HLA class I and II haplotypes in secondary DHF patients

Given the association between TNF-4 and disease severity in secondary DENV infections (Table 1), the linkage disequilibrium between TNF-4 and LTA-3 haplotypes [15], and the correlation between LTA-3 and both LTA and TNF production (Table 3), we stratified our TNF and LTA haplotype combinations or profiles (n=24) and compared these with disease severity in patients with secondary DENV infections. Two profiles, namely TNF-1,4; LTA-1,3 and TNF-1,4; LTA-3,3 were detected in patients with secondary DHF infections (10/129 or 7.8%) and controls (3/143 or 2.1%), but were entirely absent in secondary DF patients (secondary DHF vs secondary DF: Chi-square = 13.1, P=0.0003, Pc = 0.014).

We also determined the HLA class I and II alleles present in the secondary DHF patients with TNF-1,4; LTA-1,3 and TNF-1,4; LTA-3,3 haplotype profiles (Table 4). Five patients carried the HLA-B48 allele, which is relatively rare in SE Asian populations [30, see http://www.allelefrequencies.net]. Our previous analyses of HLA class I alleles in the same Thai dengue cohorts excluded relatively low frequency alleles such as HLA-B48 [26]. However, we have now recalculated the frequency of HLA-B48 in all secondary DHF patients (5.5%) and controls (1.9%) that have been HLA class I typed to date. By comparison, HLA-B48 is not detected in any of our secondary DF patients (frequency of HLA-B48 in secondary DHF vs secondary DF, Chi-Square = 10.3, P = 0.0013, Pc =0.0351). A further four patients in Table 4 also carried HLA-B57, which forms an extended haplotype with the HLA class II alleles DRB1*0701 and DQB1*03032. The most frequent HLA class II allele detected in the DHF patients shown in Table 4 was DPB1*0501, which is relatively common in SE Asians [34].

Discussion

Human genomic SNP typing is proving to be a powerful tool in disease association studies. Its application to large cohorts of Thai DENV patients has revealed significant functional allelic differences between DF and DHF in gene loci such as CD209, which encodes a DENV receptor (DC-SIGN-1) [37]. Likewise, we have previously been able to identify significant associations between certain HLA class I alleles and disease severity in Thais with secondary DENV virus infections [26]. We have now extended our analysis of the human MHC in ethnic Thais to the central or class III region, and can confirm there are considerable similarities between Thai TNF and LTA gene profiles with those reported in other mainland SE Asian populations [21]. For example, the polymorphisms that characterize the TNF-2 (-308A), TNF-4 (-238A) and LTA-2 (+249G) haplotypes [15], are all present in the major Kinh population of Vietnam at near equivalent frequencies to our Thai population controls [21] (Tables 1 and 2). These observations are also consistent with previous analyses of HLA class I and II genes in Thais, which have shown this major ethnic group to be highly representative of the large and diverse populations of mainland SE Asia [26,30,34].

A previous study of TNF -238G/A and -308G/A polymorphisms in Vietnamese DHF patients with undefined primary or secondary infections, revealed no associations with disease when compared to population controls [24]. Our study has focused on a Thai cohort stratified for sub-clinical DENV infections, primary or secondary clinical infections and disease severity (Tables 1 and 2). In these groups, which were of sufficient size to achieve at least 80% power to detect HF differences of 11-21% ($P<0.05$) in all but the primary DHF group (data not shown), we have identified a significant association between the TNF-4 haplotype (-238A) and disease severity in secondary DENV infections (Table 1). Given that both the Vietnamese and Thai populations reside in close proximity in a region where all four major DENV serotypes are in seasonal circulation, these apparent inconsistent TNF allele associations with DHF in two genetically similar ethnic groups are most likely due to differences in the design of the respective studies [24,26] (Table 1). By contrast, a preponderance of TNF-308A has been

reported in a relatively smaller study of Venezuelan DHF patients [25]. The TNF-308A allele is on the TNF-2 haplotype as defined by 3 SNPs in our study, but occurred at very similar frequencies in our patient and control groups (Table 1), which excludes this allele from association with DHF in ethnic Thais, as has been reported in the other mainland SE Asians [24]. Nevertheless, any study of highly polymorphic genes such as those encoded within the human MHC is fraught with the chance of random effects confounding observed disease associations [38]. Thus, it is imperative that studies be of sufficient size, power, and corrected to exclude confounding factors as in our current study, which should be confirmed in other cohorts of DENV exposed patients.

The -238A polymorphism that characterizes the TNF-4 haplotype has been detected in Caucasoid [15-17], African [16], Amerindian [16], as well as Oriental populations [16,21]. There is no clear evidence that the TNF-238A polymorphism directly affects TNF production, although it is located near a putative gene repressor site [39]. The TNF-238A allele has been significantly associated with other infections such as hepatitis C [22], hepatitis B [22], and severe malarial anemia [40]. However, the latter association with malaria is probably secondary to the presence of another linked TNF polymorphism (-376A), which augments TNF secretion by recruiting the OCT-1 transcription factor [13]. We did not analyze polymorphism at TNF position -376 in our study, although most individuals carrying the TNF-376A allele also tend to have the -238A allele, at least in African and European populations [13,40].

Unlike our previous studies of HLA class I polymorphisms in ethnic Thais [26], we were unable to identify any TNF or LTA associations with the specific infecting DENV serotype in either primary or secondary infections (data not shown). Nevertheless, we observed another significant association, albeit less robust, with a reduced frequency of the TNF-3 (+488A) haplotype in primary DF patients (Table 1). This haplotype could be part of, or in linkage disequilibrium with a gene locus that is protective against developing clinical disease during primary DENV exposure, hence the relatively high frequency of TNF-3 in the cohort of sub-clinical DENV infections (Table 1).

The TNF-4 haplotype has been shown to be in strong linkage disequilibrium with LTA-3 in Caucasoids [15]. Similarly, all but one of our ethnic Thai patients and controls with TNF-4 also carried LTA-3. However, none of the LTA haplotypes (including LTA-3) showed significant associations with any of our DENV patient groups (Table 2). Nevertheless, the presence of the LTA-3 haplotype correlated with *in vivo* production of both LTA and TNF, as measured by immunostaining PBMC in a relatively small subset of our patient cohort (Table 3). Most previous studies have examined the effect TNF and LTA polymorphisms *in vitro*, by measuring mitogen induced cytokine production [12-14]. By contrast, our approach provides a rare insight into host TNF and LTA haplotype associations with *in vivo* production of cytokines during the acute viraemic phase of DENV infection (Table 3), and warrants further investigation in a larger cohort of TNF/LTA genotyped patients

Two combinations of TNF-4,LTA-3 haplotypes were detected only in patients with secondary DHF and not DF (Table 4). A striking feature of these haplotypes was the prevalence of the HLA class I alleles HLA-B48 and B57 (Table 4), which occur at relatively low frequencies in ethnic Thais [30]. Both HLA-B48 and B57 are known to be in linkage disequilibrium with the TNF-238A allele on the TNF-4 haplotype [16]. HLA-B48 occurs predominantly in Oriental and Amerindian populations, but is virtually absent in Africans and Caucasoids (see www.allelefrequencies.net). HLA-B48 is also associated with a gross deletion of the non-classical MHC class I chain related (MIC) gene loci adjacent to HLA-B in the human MHC [31], which encode target ligands for natural killer (NK) cells utilizing the ubiquitous activatory lectin-like receptor NKG2D [32]. NK cells have a variety of immuno-regulatory and antiviral activities, and activation of NK cells is a feature of DENV infection in our ethnic Thai cohort

[41]. Similarly, HLA-B57, which occurs in approximately 3% of our Thai population controls [30], forms a stable haplotype with the allelic products of at least three other class II gene loci, namely DRB1*0701, DRB4*0301N and DQB1*03032 [33]. DRB4*0301N is a defective class II allele with a complete abrogation of gene expression, resulting in the HLA-DR53 null phenotype [33]. Thus, the TNF-4 and LTA-3 haplotype profiles containing either HLA-B48 or B57, which associate exclusively with severe clinical disease after secondary DENV infection (Table 4), also associate with null expression variants of other MHC encoded loci.

HLA-B48 is also one of the few class I molecules that are polymorphic in the third extracellular (alpha-3) protein domain. This is the domain that makes contact with the human CD8 co-receptor that facilitates the recognition of antigens bound to HLA class I molecules by the T cell receptor (TCR). A threonine at position 245 of HLA-B48 replaces the standard alanine found in most HLA-B alleles (see http://www.ebi.ac.uk/imgt/hla), and greatly reduces the binding of HLA-B48 to CD8 compared to alleles with Ala245 [42]. A possible consequence of this effect is HLA-B48 restricted TCRs might have evolved increased antigen binding permissiveness to compensate for the poor stabilization of the TCR/HLA-B48 complex by CD8. If this were the case, then secondary dengue infections in HLA-B48 positive individuals might result in an expanded potential for DENV serotype-cross-reactive responses.

In conclusion, our analysis of SNP-defined TNF and LTA haplotype profiles in a cohort of dengue exposed Thais has revealed TNF-specific polymorphisms that associate with severe clinical complications in patients with secondary infections (Table 1). We have evidence to suggest that certain LTA haplotypes associate with *in vivo* LTA and TNF protein production during the acute viraemic phase of dengue infection. We have also detected linkage disequilibrium between the TNF and the LTA loci that extends into the class I and II regions of the human MHC in ethnic Thais. Furthermore, some extended haplotypes that contain relatively rare HLA class I alleles, as well as HLA class II null expression variants and deletions of the non-classical MIC loci, associate uniquely with secondary DHF (Table 4). These observations suggest that a variety of gene products encoded within the human MHC may act together in determining disease outcome in immunologically primed individuals undergoing secondary exposure to DENV and are worthy of further investigation.

Acknowledgements

We thank the staff, patients and their families at Queen Sirikit National Institute of Child Health and Kamphaeng Phet Provincial Hospital, for their participation in this study. We also extend our gratitude to James Potts (University of Massachusetts, Worcester, USA) for advice and assistance on statistical analyses.

This work was supported by a Program Project grant from the National Institutes of Health USA (NIH-P01AI34533) and the US Army Medical Research and Materiel Command.

This study was granted human use approval by the Institutional Review Boards of the Thai Ministry of Public Health, the Office of the US Army Surgeon General's Office and the University of Massachusetts Medical School.

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Table 1

TNF haplotype frequencies (HF%) in sub-clinical DENV exposures, primary and secondary DF and DHF infections, and ethnically-matched healthy Thai controls

TNF Haplotype	Nucleotide Position			Sub-clinical infection N=65 HF %	Primary Infection		Secondary Infection		Control
	-308	-238	+488		DF N =59 HF %	DHF N=10 HF %	DF N=169 HF %	DHF N=132 HF %	N=143 HF %
TNF-1	G	G	G	98.5	98.3	80.0	97.0	96.2	89.5
TNF-2	A	G	G	12.3	11.9	20.0	10.1	9.8	10.5
TNF-3	G	G	A	16.9*A	3.4*A,B,C,D	30.0*B	14.2*C	11.4	12.6*D
TNF-4	G	A	G	6.2	10.2	10.0	4.1*E	15.2*E,F	7.0*F

NOTE. N= the number of patients or controls in each of the clinically defined groups. HF's are expressed as % of individuals scoring positive for a given haplotype in each defined patient group or the controls. TNF haplotype frequencies showing significant differences between patient and control groups are indicated by an asterisk

*A TNF-3 in primary DF infections vs sub-clinical infections, Chi Square = 6.0, P = 0.014, Pc = n.s., OR = 0.17 (0.03<OR<0.88).

*B TNF-3 in primary DF infections vs primary DHF, Chi Square (Yate's) = 5.5, P = 0.019, Pc = n.s., OR = 0.08 (0.01<OR<0.76).

*C TNF-3 in primary DF vs secondary DF infections, Chi Square = 5.1, P = 0.024, Pc = n.s., OR = 0.21 (0.03<OR<0.97).

*D TNF-3 in primary DF vs controls, Chi Square = 4.0, P = 0.046, Pc = n.s., OR = 0.24 (0.04<OR<1.15).

*E TNF-4 in secondary DHF vs secondary DF infections, Chi Square = 11.0, P= 0.0009, Pc=0.022 (corrected for the 4 TNF haplotypes detected and 6 patient and control groups studied, or 24 comparisons), OR = 4.13 (1.59<OR<11.17).

*F TNF-4 in secondary DHF vs controls, Chi Square = 4.7, P = 0.03, Pc = n.s. OR = 2.38 (1.01<OR<5.7).

Table 2

LTA haplotype frequencies (HF%) in primary and secondary DF and DHF infections, and ethnically-matched healthy Thai controls.

	Nucleotide Position			Primary Infection		Secondary Infection		Controls
LTA Haplotype	+249	+365	+720	DF N =59 HF%	DHF N=10 HF%	DF N=163* HF%	DHF N=129* HF%	N=143 HF%
LTA-1	A	C	C	42.4	70.0	57.1	56.6	49.0
LTA-2	G	G	A	62.7	70.0	66.3	67.4	63.6
LTA-3	A	G	C	54.2	40.0	44.8	43.4	51.0

NOTE. N= the number of patients or controls in each of the clinically defined groups. HF's are expressed as % of the number patients in each defined patient group or the controls.

* Six patients with secondary DF, 3 with secondary DHF and 65 sub-clinical dengue infections that were TNF genotyped in Table 1 were not LTA genotyped (see Materials and Methods).

Table 3

LTA haplotypes associated with *in vivo* intra-cellular cytokine production during the acute phase of dengue viraemia

LTA haplotype	Cytokine	Immunostaining Positive n/N (%)	Immunostaining Negative n/N (%)	Fisher's Test P=	Pc=
LTA-2	TNF	4/9 (44.4%)	8/8 (100%)	0.029	n.s.
LTA-3	TNF	7/9 (77.8%)	2/8 (25%)	0.044	n.s.
LTA-3	LTA	6/7 (85.7%)	3/10 (30%)	0.036	n.s.

NOTE. N = the total number of patients scoring positive or negative for either intracellular LTA or TNF cytokine production; n = the number of patients with the given LTA haplotype, which is expressed as a % of N in parentheses

Table 4

Extended MHC haplotypes detected only in Thai patients with secondary DHF.

Patient disease severity	MHC region						
	class I		class III (central)		class II		
	HLA-A	HLA-B	TNF	LTA	DRB1*	DQB1*	DPB1*
DHF-2	2, 11	56, 60	**1, 4**	**1, 3**	*1502,*1602	*0501,*0502	*0402,*0501
DHF-2	2, 24	38, **48**	**1, 4**	**1, 3**	*0405,*1401	*0402,*0502	*0501,*2201
DHF-2	1, 2	**57**, 75	**1, 4**	**1, 3**	***0701,***1202	*0301, ***03032**	*0401,*0901
DHF-3	1, 11	**57**, 60	**1, 4**	**1, 3**	***0701,***1502	***03032,***0501	*0901,*1301
DHF-1	33, 34	**57**, 75	**1, 4**	**3, 3**	***0701,***1502	***03032,***0601	*0501,*0501
DHF-2	2, 24	**48**, 62	**1, 4**	**3, 3**	*1501,*1405	*05031,*05031	*0201,*0501
DHF-2	2, 24	13, **48**	**1, 4**	**3, 3**	*1101,*1602	*0301, *0502	*0501,*0501
DHF-2	11, 24	**48**, 75	**1, 4**	**3, 3**	*1101,*1502	*0301, *0501	*0501,*1301
DHF-2	3, 24	38, **57**	**1, 4**	**3, 3**	***0701,***1502	***03032,***0502	*0201,*0402
DHF-3	2, 2	39, **48**	**1, 4**	**3, 3**	*0405,***0405	*0302, *0302	*0402,*0501

NOTE. DHF disease severity grades 1-3 are defined according to WHO criteria [2]. SNP-defined TNF and LTA haplotype profiles detected only in patients with secondary DHF, and relatively rare HLA alleles and haplotypes associated with null expression of MHC-encoded proteins [31,33], are given in bold.