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Dengue Virus Specific T Cell Responses to Live Attenuated Monovalent Dengue-2 and Tetravalent Dengue Vaccines

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SUMMARY **The proliferative T cell responses to dengue vaccines were studied using the parental strains of dengue vaccines as antigens in 26 dengue immune individuals who resided in Bangkok which is the endemic area of dengue infection. The magnitude of the T cell responses in subjects with flavivirus cross-reactive neutralizing antibody was much higher and the cross-reactivity was broader than in those with dengue serotype-specific neutralizing antibodies, Japanese encephalitis (JE) specific antibodies or dengue cross-reactive antibodies. The T cell response in those with neutralizing antibody against a single serotype or in those who had dengue cross-reactive neutralizing antibody was relatively low, independent of the level or degree of cross-reactivity of the antibody. Evaluation of the proliferative T cell responses in 8 recipients of the monovalent dengue-2 (16681-PDK53) or the tetravalent dengue vaccines demonstrated that both vaccines induced high levels of neutralizing antibody as well as high levels of T cell responses to all serotypes of dengue virus. These results indicate that the evaluated dengue vaccines efficiently induced humoral and cell mediated immunity comparable to natural infection with dengue virus.**

Dengue hemorrhagic fever/ dengue shock syndrome (DHF/ DSS) is a life-threatening disease affecting children in many areas of the world, including Southeast Asia and Central and South America.[1] Epidemiological and clinical studies have revealed that DHF/DSS occurs at a much higher incidence in patients with secondary dengue virus infection than in those with primary infection.[2] To prevent DHF/DSS, live attenuated vaccines against all four serotypes of dengue virus were developed and evaluated by investigators at Mahidol University in Thailand. Immunization with the monovalent dengue-2 (16681-PDK53) vaccine was shown to be safe and highly immunogenic.[3,4] In human volunteers, the dengue-2 vaccine induced neutralizing antibody which persisted for at least 2 years following immunization.[4] Immunization with any of the other three monovalent vaccines, i.e. dengue-1, dengue-3 or dengue-4 vaccine was also found to be safe and induced seroconversion in all volunteers.[5] Tetravalent dengue vaccine comprising all four serotypes of dengue viruses, namely, dengue-1 (16007-PDK13), dengue-2 (16681-PDK53), dengue-3 (16562-PGMK30/F3) and dengue-4 (1036-PDK48) has been developed. Immunogenicity studies of the vaccine in children aged 10-14 years, 5-9 years and a small number of children of 1-4 years of age have all shown that the vaccine was safe and highly immunogenic in inducing seroconversion in nearly 80% of the vaccinees. The neutralizing antibody titers persisted for 3 years after immunization.[5] In addition, T cell responses were studied in individuals following immunization with live attenuated dengue vaccines. Memory T cell response was demonstrated in immune naive American volunteers

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who received the monovalent dengue-2 (16681-PDK53) vaccine. Both type-specific and cross-reactive memory T cell responses to live viruses and noninfectious antigens derived from the prototype strains of dengue viruses were observed.[6]

The present study was undertaken to evaluate the T cell responses to the dengue vaccine viruses in immune individuals in an endemic area by using the parental strains of Mahidol University dengue vaccines as antigens. In this study, the T cell response to dengue vaccine of peripheral blood monocuclear cells (PBMC) obtained from dengue immune individuals and dengue vaccine recipients was examined following *in vitro* stimulation by live viruses and noninfectious antigens prepared from the parental strains of dengue vaccines. The parental strains of dengue vaccines had only a few nucleotide substitution in the genomes as compared to its vaccine derivative.[7] This approach allowed the use of almost identical antigen preparation as the vaccine viruses and contained high viral titers. LLC-MK2 cells, derived from rhesus monkey kidney, were used to avoid the stimulation by mosquito cell antigen that may have increased the background of T cell responses because most of the Thai population are constantly exposed to mosquitoes. The patterns of proliferative T cell responses obtained from these subjects were analyzed using two forms of antigen: live dengue virus antigens and noninfectious dengue antigens prepared in LLC-MK2 cells. The live virus antigens had been shown to induce both CD4+ and CD8+ T cells,[8] while the noninfectious antigens induce predominantly CD4+ T cells.[9]

This study demonstrated that the responses in immune individuals were detectable and generally similar to the findings previously done using the prototype strains of dengue viruses. The responses were measured in individuals receiving monovalent dengue-2 (16681-PDK53) vaccine as well as in those receiving the tetravalent dengue vaccines. Although all vaccine receipients were initially tested seronegative, some were subsequently shown to be immune prior to vaccination. This provided a rare opportunity to observe the responses in immune individuals that unknowingly have been exposed to natural infection with dengue virus prior to vaccination.

MATERIALS AND METHODS

Subjects

Twenty-six consenting healthy Thai male volunteers, aged 18-20 years old who resided in Bangkok, where dengue infection is common,[10,11] were enrolled to this study. The evidence of immunity from natural infection with dengue virus was demonstrated using an assay of hemagglutination inhibiting antibody to dengue viruses. A clinical history of DHF was found in 6 individuals and a history of Japanese encephalitis (JE) vaccination was found in 3 individuals in this group. All subjects had received no medication for one month prior to the study and had no underlying diseases. Thirty mililitres of blood were taken from each volunteer; sera were tested for antibody to dengue viruses by hemagglutination inhibition test (HI) and plaque reduction neutralization test (PRNT), as previously described,[12,13] and PBMC were used for T cell study.[9]

In addition, eight male volunteers (5 and 3), age 18-35 years old, who were residents of Bangkok, were immunized with dengue-2 (16681-PDK53) vaccine and the tetravalent dengue vaccines, respectively. This study was a part of the phase I trial of the vaccines conducted by Center for Vaccine Development, Mahidol University. The trial was approved by the Ethical Review Committee, Ministry of Public Health, Nonthaburi, Thailand. Written informed consent was obtained from all subjects. The volunteers were tested seronegative to dengue viruses serotypes 1-4, or JE virus at the time of enrollment and the vaccine inoculation was performed within 3 months. Sixty mililitres of blood were taken from each volunteer just prior to virus vaccine inoculation (day 0) and at 60 days after vaccination. Sera were tested for antibodies to the dengue viruses and PBMC was isolated for the T cell study.

Dengue specific antibodies

Antibodies to dengue viruses were determined by HI and PRNT, as previously described.[12,13] Dengue specific IgM and IgG were determined using dengue fever virus IgM capture and IgG ELISA (MRL Diagnostics, Cypress, CA, USA) as described by the manufacturer. Positive antibody was indicated when the Index Value was >1.00.

Live virus antigens

Dengue-1 (16007), dengue-2 (16881), dengue-3 (16562), dengue-4 (1036) viruses, which were the parental strains of dengue vaccines, were used as antigens for T cell study. JE (Beijing strain) virus was obtained from the Armed Forces Research Institute of Medi-

cal Sciences (AFRIMS), Bangkok, Thailand. The viruses were propagated in rhesus monkey kidney (LLC-MK2) cells. The virus stock was prepared from infected cell culture supernatant fluid after centrifugation,[9] and aliquots were stored at -70°C until use. The virus titer was determined by a plaque assay in LLC-MK2 cells as previously described.[14] The viruses used in this study had titers of approximately 10^7 plaque forming units (pfu)/ml.

Noninfectious virus antigens

Dengue and JE virus antigens were prepared from sonicated glutaraldehyde-treated, dengue vaccine parental strains or JE virus infected LLC-MK2 cells as previously described[9] with same modification in which LLC-MK2 cells were used instead of C6/36 mosquito cells. Control antigen was prepared from uninfected LLC-MK2 cells by a similar method.

Proliferative T cell response

PBMCs from the healthy volunteers were isolated by Ficoll-Hypaque density gradient[15] and immediately used in lymphoproliferation assay. PBMCs obtained from the dengue vaccine recipients were isolated and aliquots of PBMCs from each donor were cryopreserved in RPMI 1640 containing 40% fetal calf serum (FCS) and 10% dimethyl sulfoxide (Sigma Chemical, St. Louis, MO, U.S.A.) in liquid nitrogen until use. The PBMCs with at least 90% viability by trypan blue dye exclusion, were recovered and used in this study.

Triplicate cultures of PBMCs (1.2×10^5 cells) in AIM-V medium (GibcoBRL) containing 10% heat inactivated non-immune human sera were incubated with dengue antigens in 96-well round-bottom tissue culture plates (Costar, Cambridge, MA, USA) at 37°C for 6 days. Live dengue-1,-2,-3, and -4 viruses, JE virus, and uninfected LLC-MK2 cell supernatant were two-fold serially diluted from 1:4 to 1:512. The cultures were pulsed with 1.0 μCi of [^{3}H] thymidine (NEN™ Life Science, Boston, MA, USA) on day 6 for 8 hours before harvest. The cells were harvested and the tritiated thymidine incorporation was determined from the supernatant using a liquid scintillation counter (Rack beta II, Wallac Oy, Finland).

Dengue virus-specific proliferative responses were determined using the maximal response with each virus. Data are presented as relative stimulation index (RSI) = mean cpm of cultures stimulated with dengue antigen - mean cpm of cultures with medium alone/mean cpm of cultures stimulated with uninfected cell antigen - mean cpm of cultures with medium alone.[16]

RESULTS

Proliferative T cell responses to dengue vaccine viruses in immune individuals after natural infection

The T cell response was examined in a total of 26 immune individuals who resided in Bangkok, a dengue virus endemic area.[10,11] The antibody response was determined using HI and PRNT for dengue serotypes 1, 2, 3, and 4 and JE viruses. Based on these results, these individuals can be divided in to 4 groups: 8 with dengue serotype-specific antibodies, 3 with JE-specific antibodies, 6 with dengue cross-reactive antibodies, 9 with broad flavivirus cross-reactivity. The T cell responses of PBMCs from these individuals were examined following *in vitro* stimulation by live dengue viruses and noninfectious dengue antigens prepared from the parental strains of dengue vaccines (Table 1).

Eight individuals had dengue specific neutralizing antibodies to a single serotype of the virus, of which 1, 3, 3 and 1 had responses to dengue serotypes 1, 2, 3, and 4, respectively. Six of these 8 subjects demonstrated proliferative T cell responses to live viruses as well as to noninfectious virus antigens. The magnitude of the T cell response was generally low, with a relative stimulation index of less than 10, except in one individual who had antibodies to only dengue serotype 4 and T cell responses against dengue serotypes 3 and 4 (RSI of 12 and 16, respectively). The serotype specificity of T cell responses after stimulation with live viruses was different from the response after stimulation with noninfectious virus antigens. Among individuals who had serotype specific neutralizing antibodies to dengue-1 or -2 or -4 virus, the highest T cell responses to noninfectious virus antigens were against the same dengue serotypes. Therefore, a correlation was found between the patterns of T cell responses to noninfectious virus antigens and the patterns of neutralizing antibodies in this group. This correlation was not observed in the responses to live dengue viruses.

Among the 3 individuals who had JE-specific antibody, no T cell responses that cross-reacted to dengue virus were observed with the exception of one individual who showed some response to noninfectious dengue-1 and -2 antigens at low level (RSI = 3).

Table 1 Proliferative T cell responses after stimulation with live virus antigens and noninfectious virus antigens in naturally immune individuals

Subject	History of DHF	Dengue specific antibodies										Memory T cells (Maximal relative stimulation index)							
		PRNT					Reciprocal HI titer					Live virus antigens				Noninfectious virus antigens			
		D1	D2	D3	D4	JE	D1	D2	D3	D4	JE	D1	D2	D3	D4	D1	D2	D3	D4
Dengue serotype specific																			
1	-	*+	**-	-	-	-	10	-	-	-	-	3	1	2	1	3	1	1	1
2	-	-	+	-	-	-	-	20	-	-	-	1	1	3	1	2	8	1	2
3	-	-	+	-	-	-	-	10	-	-	-	1	1	1	1	1	1	1	1
4	-	-	+	-	-	-	-	10	-	-	-	1		1	1	1	1	1	1
5	-	-	-	+	-	-	10	-	80	10	10	6	5	2	5	1	1	1	1
6	-	-	-	+	-	-	-	-	80	10	10	1	1	3	1	4	5	1	1
7	-	-	-	+	-	-	-	-	80	10	-	1	3	3	1	3	3	2	1
8	-	-	-	-	+	-	-	-	-	10	10	6	5	5	5	3	3	12	16
JE specific																			
9	-	-	-	-	-	+	-	-	-	10	80	1	1	1	1	2	1	1	1
10	-	-	-	-	-	+	-	-	-	-	20	1	1	1	1	3	3	1	1
11	-	-	+	-	-	+	-	-	-	-	10	1	1	1	1	1	2	1	1
Dengue cross-reactive																			
12	+	+	+	+	+	-	80	160	320	160	160	3	1	3	1	2	2	1	1
13	+	+	+	+	+	-	80	160	80	80	160	6	1	5	1	4	1	4	1
14	-	+	+	+	+	-	40	160	40	320	80	1	1	3	1	2	3	1	2
15	-	+	+	+	+	-	20	80	20	10	10	4	1	3	2	9	5	1	1
16	-	+	+	+	+	-	10	80	-	40	40	1	3	1	1	1	1	1	1
17	-	+	+	+	-	-	10	-	-	-	-	3	1	1	3	1	1	1	1
Flavivirus cross-reactive																			
18	-	+	+	+	+	+	160	320	160	320	160	16	6	13	6	20	2	26	26
19	-	+	+	+	+	+	80	80	320	320	80	4	3	3	1	4	4	5	1
20	-	+	+	+	+	+	80	80	320	320	40	3	3	1	1	4	3	7	5
21	+	+	+	+	+	+	80	160	80	160	320	7	7	6	3	2	3	3	1
22	+	+	+	+	+	+	80	80	160	320	160	4	3	3	1	8	8	7	3
23	+	+	+	+	+	+	80	160	160	80	80	4	6	4	1	6	7	8	9
24	+	+	+	+	+	+	80	160	80	80	40	6	1	1	3	17	9	13	3
25	-	+	+	+	+	+	40	160	20	40	20	7	3	4	3	13	4	3	3
26	-	+	+	+	+	+	10	10	10	40	20	4	3	6	2	4	3	1	2

HI, hemagglutination-inhibition test; PRNT, plaque reduction neutralization test; Viruses: D, dengue; JE, Japanese encephalitis; C, cell control; Ag, antigen.
*- = positive result, **+ = negative result

Table 2 T cell responses after stimulation with live virus antigens and noninfectious virus antigens in individuals receiving candidate dengue vaccines.

Subjects	Day	Dengue specific antibody												Memory T cell (Maximal relative stimulation index)									
		PRNT					HI					ELISA (Index value)		Live viral antigens					Noninfectious viral antigens				
		D1	D2	D3	D4	JE	D1	D2	D3	D4	JE	IgM	IgG	D1	D2	D3	D4	JE	D1	D2	D3	D4	JE
Dengue-2 vaccine recipients																							
#1	0	-	-	-	-	-	-	-	-	-	-	0.80	0.16	1	2	1	2	1	1	2	1	2	1
	60	-	31	-	-	-	-	20	-	-	-	2.45	0.80	6	9	5	4	5	5	14	6	10	4
#2	0	-	-	-	-	-	-	-	-	-	-	0.80	0.80	1	2	2	1	3	2	1	1	2	2
	60	39	180	80	35	78	80	160	80	80	160	0.46	3.08	12	20	14	14	13	11	14	9	3	7
#3	0	-	-	-	-	-	-	-	-	-	-	0.45	0.71	7	6	8	13	6	2	1	1	1	1
	60	-	320	10	-	-	-	160	-	-	-	9.59	1.47	36	45	15	15	3	6	8	4	5	1
#4	0	-	-	-	-	14	-	-	-	-	-	0.99	0.84	6	5	6	6	3	1	2	1	1	3
	60	21	230	68	-	21	320	320	80	80	2,560	1.57	3.40	19	18	10	2	16	3	3	6	1	3
#5	0	-	-	-	-	-	-	-	-	-	-	0.53	1.31	4	1	2	1	1	7	5	7	10	6
	60	260	460	110	27	-	40	80	20	40	320	0.89	3.9	17	18	19	16	16	3	10	6	10	1
Tetravalent dengue vaccine recipients																							
#6	0	-	-	-	-	-	-	-	-	-	-	0.77	0.24	2	1*	2	1	1	2	1	2	1	1
	60	14	190	-	140	-	-	40	-	-	-	2.97	1.27	5	9	3	10	2	5	2	5	1	1
#7	0	-	-	-	-	-	-	-	-	-	-	1.09	4.04	2	1	6	2	3	1	1	5	4	4
	60	500	480	3600	30	150	640	1280	640	640	640	1.13	9.04	5	7	6	2	4	13	13	10	3	8
#8	0	-	20	12	15	-	-	-	-	-	-	0.66	5.89	1	1	1	1	1	2	1	1	1	1
	60	370	800	480	150	22	1,280	640	1,280	2.560	1,280	1.18	8.27	5	3	5	5	1	7	7	8	10	5

The data are presented as RSI of day 0 and day 60 PBMCs of each subject.

The six individuals who had dengue virus-cross-reactive neutralizing antibodies had a T cell response to at least one live dengue virus while only half of them responded to the noninfectious virus antigens. Among those that responded to the live viruses, four individuals showed cross-reactive responses to 2 virus serotypes, and dengue-1 and dengue-3 were the most common viruses that stimulated T cells in this group, while the other two individuals showed a response to only one serotype. The magnitude of the responses of the subjects in this group was generally low, compared to the responses of the first group. The T cell responses in the 2 donors with a history of DHF were similar, both in terms of magnitude and serotypic patterns to the others in the same group. In addition, no correlation between the magnitude of antibody measured by HI antibody titer and the magnitude of T cell responses was observed.

Higher levels of the T cell responses to both live viruses and noninfectious antigens were observed in 9 individuals who had flavivirus cross-reactive neutralizing antibodies. The patterns of T cell responses after stimulation with either live viruses or noninfectious antigens were mainly cross-reactive, although the serotypic patterns of the T cell response in each individual to live viruses were different from the response to noninfectious antigens. In contrast to the other groups, the magnitude of T cell responses in this group to noninfectious antigens was higher than that to live dengue viruses. Dengue-1 was the most common and dengue-4 was the least common viruses that stimulated T cells in this group. In addition, no correlation between the magnitude of antibody response measured by HI and the magnitude of T cell responses was observed. The T cell responses of the 4 individuals with history of DHF and the other 5 individuals without history of DHF were also similar.

T cell responses in dengue vaccine recipients

The T cell response in 5 individuals receiving the monovalent dengue-2 (16681-PDK53) vaccine and 3 individuals receiving the tetravalent dengue vaccines was characterized (Table 2). These individuals were seronegative at the time of entry to the study, as determined by HI to dengue serotypes 1, 2, 3, and 4. Sera obtained from day 0 and day 60 following immunization were also examined using HI and PRNT for dengue serotypes 1, 2, 3, and 4 and JE virus, and dengue specific IgM and IgG antibodies using commercial ELISA. The T cell responses of PBMCs from these individuals were examined following stimulation by live dengue viruses and noninfectious dengue antigens derived from the parental strains of the dengue vaccines.

Each of the 5 individuals who received dengue-2 vaccine exhibited different patterns of responses. Volunteers #1 and #2 were truly non-immune and had no pre-existing antibodies detected by HI, PRNT, IgM, and IgG ELISA to dengue viruses. They had minimal or no T cell response on day 0. At day 60 post immunization with live attenuated dengue-2 vaccine, #1 developed dengue-specific IgM antibodies as well as dengue-2 specific antibodies detected by HI and PRNT. On the other hand, #2 developed dengue-specific IgG, but no IgM antibodies as well as flavivirus cross-reactive antibodies detected by HI and PRNT. Both #1 and #2 developed flavivirus cross-reactive T cell response to the live viruses and to the noninfectious antigens of all four serotypes of dengue viruses and to JE virus.

Volunteers #3 and #4 were also non-immune by the criteria of having no pre-existing antibodies detected by HI, PRNT, IgM, and IgG ELISA to dengue viruses, although #4 had a minimal level of neutralizing antibodies to JE virus. However, both demonstrated pre-existing memory T cell response to live dengue and JE viruses but not to noninfectious antigens on day 0. At day 60 post immunization with the live attenuated dengue-2 vaccine, both developed dengue-specific IgM and IgG antibodies, but #3 developed dengue-2 specific antibodies detected by HI and PRNT, while #4 developed flavivirus cross-reactive HI and PRNT antibodies. However, both of them developed flavivirus cross-reactive T cell responses to live flaviviruses and also to noninfectious antigens of all four serotypes of dengue viruses and JE virus.

Volunteer #5 was not truly non-immune, by having detectable dengue specific IgG, although no pre-existing antibody was detected by HI, and PRNT. This individual had pre-existing flavivirus cross-reactive memory T cells detected primarily by stimulation with noninfectious antigens. After receiving the dengue-2 vaccine, this volunteer developed a secondary antibody response as well as flavivirus cross-reactive T cell response.

These data demonstrate that each of the five individuals with different patterns of pre-existing immune status against

dengue viruses developed different patterns of antibody responses to dengue virus after receiving monovalent dengue-2 vaccine, either flavivirus-cross-reactive or serotype-2 specific responses. All five vaccinees developed dengue virus specific T cells to both live viruses and noninfectious antigens. The patterns of T cell response to both antigens were mainly cross-reactive, however, the highest response was against dengue-2 virus. Of note, subject #1 and #4 developed fever with epistaxis or petichial hemorrhage, respectively, 7 days after vaccination, clinically classified as grade II DHF.[17] The magnitude of T cell responses in these two individuals were not different from the others.

Among the three individuals who were recipients of the tetravalent vaccines, only #6 was truely seronegative at the time of recruitment in this study. After immunization with the tetravalent vaccine, this individual developed both dengue specific IgM and IgG antibodies, as well as high neutralizing antibodies to dengue-2 and -4 and also to dengue-1. #6 developed T cell responses to all four serotypes of live dengue viruses, predominantly serotypes-2 and -4.

Vaccinee #7 was seronegative by both HI and PRNT; however, the same serum was found to have positive dengue specific IgM and IgG as well as detectable T cell to dengue-3 and JE live virus and noninfectious antigens. Volunteer #8 who was seronegative by HI test was subsequently found to have dengue specific IgG on day 0 as well as having low levels of neutralizing antibodies against dengue-2, -3, and -4 at the time of vaccination. After receiving the tetravalent vaccine, both #7 and #8 developed high titer neutralizing antibodies against all four serotypes of dengue viruses as well as to JE virus. In addition, flavivirus cross-reactive T cell responses to the live virus as well as the noninfectious antigens were demonstrated in both individuals at day 60 after immunization with the tetravalent vaccine. In summary, all three recipients developed cross-reactive T cell responses to live viruses and also to the noninfectious antigens, independent of their pre-existing immune status. None of them developed DHF following vaccination.

DISCUSSION

In this study, the T cell response of PBMC to dengue vaccine in dengue immune individuals and dengue vaccine recipients was examined following *in vitro* stimulation by live viruses and noninfectious antigens prepared from the parental strains of dengue vaccines. The study demonstrates varying magnitudes and cross-reactivity patterns of the T cell responses in the immune individuals who had been exposed to dengue viruses by natural infection. A low level of T cell response with the highest response against the same serotype of virus that induced neutralizing antibodies was observed in dengue-1, -2, and -4 immune individuals. These findings were similar to those observed in previous studies using different strains of dengue viruses.[9,18,19] These individuals with dengue serotype specific neutralizing antibodies presumably had previous natural infection by a single serotype of dengue virus. A similar magnitude of T cell responses was also observed in immune individuals with dengue cross-reactive neutralizing antibodies, despite the much higher level and also more cross-reactive HI antibodies. These individuals were suspected of having been infected with more than one episode or with more than one serotype of the virus.

On the other hand, a much stronger T cell response was observed in those who had flavivirus cross-reactive neutralizing antibodies, compared to those with dengue cross-reactive antibodies. These findings raise a question on the role of other flaviviruses, such as JE, in the T cell response to dengue viruses. In this study, JE immune subjects did not appear to have a significant level of cross-reactive T cells to dengue viruses. A recent study in mice demonstrated similar findings: cytotoxic T lymphocyte clones isolated from dengue virus-immunized mice did not recognize the homologous peptides of the Japanese encephalitis virus.[20] Therefore, it is unlikely that prior exposure to JE virus will prime memory T cells that may cross-react with dengue viruses and be responsible for the high magnitude T cell responses in subjects with flavivirus cross-reactive neutralizing antibodies. Further studies are needed to clarify this issue.

In addition, no differences in the T cell responses of six subjects who had a history of DHF were detected, in terms of both the magnitude and the serotypic patterns when compared with those who had no history of DHF. The T cell response in the two subjects with dengue cross-reactive neutralizing antibodies was much lower than in the other four with flavivirus cross-reactive neutralizing antibodies. This finding demonstrated that the level of the T cell response can be either high or low in individuals after having DHF some years later. It is not known whether these individuals had a high level of T cell response at the time of DHF.

The study of T cell responses was extended to 8 individuals who were recipients of the monovalent dengue 2 vaccine and the tetravalent dengue vaccine. These individuals represented a small percentage of the adult population in Thailand who were seronegative to dengue virus. Thirteen percent of young adults in Bangkok were seronegative to dengue virus as determined by HI and neutralization.[10] The large amount of blood required for this study ruled out the extension to more vaccinees. However, the data obtained from a small number of individuals in this study provided several insights into the nature of cell-mediated immunity in the real situation in an endemic area of dengue virus. First, individuals in the endemic area were frequently exposed to dengue viruses and developed immunity from natural infection. The immunity can be demonstrated by the seroconversion and/or by a proliferative response of T cells after *in vitro* stimulation with dengue viruses. Two individuals in this study had detectable dengue specific T cells without detectable antibodies determined by all three assays. Second, the monovalent dengue-2 (16681-PDK53) was able to induce high levels of neutralizing antibodies as well as high level of T cell responses to all serotypes of dengue viruses in this study. The pattern of the T cell response was cross-reactive flavivirus with the highest response against dengue-2 virus. This observation can be seen in individuals who exhibited cross-reactive neutralizing antibodies as well as in those who developed a dengue-2 specific neutralizing antibody response. Third, the T cell response in individuals who developed DHF following vaccination was not different in terms of the magnitude nor the serotypic patterns of the antigen prepared from the whole viruses used in this study. Vaccinee #1 developed DHF despite having all immunologic evidences of primary dengue infection. Vaccinee #4 developed DHF with no evidence of pre-existing antibodies to dengue viruses, except having detectable T cells to all four dengue serotypes and a minimal level of neutralizing antibody to JE virus. Last, the tetravalent dengue vaccines were able to induce high levels of neutralizing antibodies against all serotypes of dengue viruses in all vaccinees. The T cell response can be detected with similar patterns and magnitudes as in the recipients of the dengue-2 monovalent vaccine.

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