Report of Four Volunteers with Primary, Secondary and Tertiary Dengue Infections during a Prospective Cohort Study

Herman Kosasih[a], Hadi Yusuf[b], Primal Sudjana[b], Bachti Alisjahbana[b], Suharyono Wuryadi[c], Akterono[a], Ratna Irsiana Tan[a], Djoko Yuwono[c], Charmagne Beckett[d], Kevin R. Porter[d] and Patrick J. Blair[a]✉

[a]*Viral Diseases Program, U.S. Naval Medical Research Unit #2, American Embassy FPO AP 96520-8132 Jakarta Indonesia*

[b]*Internal Medicine, Hasan Sadikin Hospital, Jl Pasirkaliki 190, Bandung, Indonesia*

[c]*National Institute of Health Research and Development, Ministry of Health, Jl Percetakan Negara No. 29, Jakarta Pusat 10560, Indonesia*

[d]*Naval Medical Research Center, Silver Spring, MD, USA*

Abstract

A prospective study of dengue fever (DF) and dengue haemorrhagic fever (DHF) in adults was conducted at two factories in Bandung, Indonesia, between August 2000 and July 2004. A total of 2978 employees were followed for the development of fever and clinical signs of DF/DHF. Among 1431 patients reporting a febrile illness, dengue infections were detected in 177 individuals. Four enrollees with evidence of previous dengue exposure experienced two consecutive episodes of dengue infection. Analysis of pre-illness sera revealed that one patient had neutralizing antibodies (nAbs) to DENV-1, two had nAbs to DENV-2, and another had nAbs to DENV-2, 3 and 4. The individual with pre-illness neutralizing antibodies to DENV-1 experienced a DENV-3 secondary infection, followed by a third infection with an unknown serotype. One of the two individuals with pre-illness neutralizing antibodies to DENV-2 experienced a DENV-3 and then a DENV-1 infection. The other DENV-2 immune patient experienced sequential infections with DENV-4 and DENV-3 viruses. Finally, the individual with pre-illness neutralizing antibodies against three dengue viruses had a subsequent infection with DENV-4, followed one year later by DENV-3. In this instance, the patient acquired grade II DHF (WHO criteria) from the subsequent DENV-4 infection. These data provide confirmatory evidence that humans can experience three sequential heterologous dengue infections. Importantly, the occurrence of a second and third infection in individuals with pre-illness antibodies against multiple dengue serotypes indicates that neutralizing antibodies are cross-reactive *in vitro* but not cross-protective *in vivo*.

Keywords: Multiple, dengue, infection.

Introduction

Dengue fever (DF) and dengue haemorrhagic fever (DHF) are endemic diseases that occur with regularity across Indonesia. The first recognized outbreak of DHF occurred during the 1960s. Since then, outbreaks have occurred both in rural and major urban areas

✉*blair@namrutwo.med.navy.mil;* ☎ *62-21-421-4457; Fax: 62-21-428-00819*

across the archipelago. In recent years, an increase in dengue cases among individuals ≥15 years old had been reported[1,2] but the clinical manifestations of dengue disease progression are poorly understood in adults. To further evaluate the epidemiology of DF and DHF in an adult population, a prospective dengue cohort study was initiated in adult workers at two textile factories in Bandung, West Java, Indonesia.[3] During the course of this study, enrollees were actively followed up for the development of the disease over a four-year study period.

Methods

After obtaining informed consent, 2978 workers were enrolled and medical history questionnaires and demographic data forms completed. The enrollees underwent physical examination and their blood samples were taken. Baseline neutralizing antibody titers to assess their previous dengue exposure in addition to routine laboratory blood counts and chemistries were measured. When a volunteer experienced a febrile illness, a medical evaluation was conducted and blood samples were taken for complete blood counts (CBC), DENV isolation, reverse-transcriptase polymerase chain reaction (RT-PCR) and serology (anti-DENV IgM, haemagglutination-inhibition/HI, and neutralizing antibodies). Volunteers with thrombocoytopenia (platelet count ≤100 000/mm^3) or those appraised as severely ill, were hospitalized. During their hospital stay, haematocrit and platelet counts were evaluated daily, while chest and abdominal sonograms were performed to detect plasma leakage. Convalescent sera were collected from hospitalized and non-hospitalized patients on day 10 through day 14. Serosurveys were conducted every three months to obtain peripheral blood mononuclear cells (PBMCs) and plasma for the evaluation of dengue immune status. Fifty per cent plaque reduction neutralizing antibody titers were determined by probit analysis. Titers below a dilution of 1:20 on a PRNT were considered negative.

An enrollee was considered to have a DENV infection if he or she had a compatible clinical illness and laboratory evidence of DENV, which included either identification of the infecting virus by RT-PCR or virus isolation, the presence of IgM antibodies, or a four-fold increase in HI antibody titer. World Health Organization (WHO) criteria were used to categorize disease status.[4]

Results and discussion

From August 2000 until July 2004, 1431 febrile illnesses were evaluated, which resulted in the confirmation of 177 acute dengue infections. Among these, four dengue-immune enrollees experienced two separate (consecutive) episodes of dengue infection.

The first instance of multiple dengue infection occurred in a 42-year-old male who presented to the hospital with fever, headache, myalgia, coryza, nausea and vomiting (Table 1). Analysis of pre-illness serum obtained 2 months prior to his second dengue infection revealed the presence of neutralizing antibodies to DENV-1 ($PRNT_{50}$ titer: 187). The neutralizing antibody profile of the subsequent convalescent serum indicated that the infecting serotype was DENV-3. Five months later, the study volunteer demonstrated anti-DENV IgM antibodies and a four-fold increase in HI antibody titers suggesting a third dengue infection (Table 2). However, in this instance, the infecting serotype was not determined. While both episodes required hospitalization, the first episode was slightly more severe with the onset of thrombocytopenia. Clinically, both episodes were diagnosed as DF.

Table 1: Laboratory and clinical results following second dengue infection

Enrollee ID	Sex	Age	Second infection															Diagnosis
			Lab results										Clinical Manifestations					
			PRNT 50% Titer				IgM ELISA		HI		RT-PCR	Virus isolation	Symptoms & sign	Haemorrhagic tendencies	Thrombo-cytopenia	Plasma leakage	Circulatory failure	
			Serotypes	Pre-illness	Acute	Conv.	Acute	Conv.	Acute	Conv.								
730	M	42	DENV-1 DENV-2 DENV-3 DENV-4	187 <10 12 <10	<10 39 13 <10	>25 000 1463 6870 56	0.5	4.8	<10	640	Negative	Negative	Fever, headache, myalgia, coryza, nausea, vomiting, diarrhea, leukopenia	No	Yes	No	No	Dengue fever
1793	F	29	DENV-1 DENV-2 DENV-3 DENV-4	<10 359 12 11	<10 165 10 <10	3402 >1000* 6024 490	0.7	10.9	160	10 240	DENV-3	Negative	Headache, retro-orbital pain, myalgia, sore-throat, nausea, vomiting	Yes	No	No	No	Dengue fever
2159	M	38	DENV-1 DENV-2 DENV-3 DENV-4	10 215 <10 <10	<10 145 <10 <10	139 3337 301 493	0.17	1	<10	2560	DENV-4	DENV-4	Fever, headache, myalgia, nausea	No	No	No	No	Dengue fever
2119	F	30	DENV-1 DENV-2 DENV-3 DENV-4	<10 124 99 29	<10 133 <10 <10	567 7404 1029 5414	0.2	1.1	10	2560	Negative	DENV-4	Fever, headache, retro-orbital pain, myalgia, nausea, vomiting, abdominal pain	Petechiae, nose bleeding, gum bleeding, tourniquet test	Yes	Pleural effusion, ascites, hypo proteinemia	No	Dengue haemorrhagic fever gr.2

*No plaques were detected even in the highest dilution (1:1000)

Table 2: *Laboratory and clinical results following third dengue infection*

Enrollee ID	Sex	Age	Third infection															Diagnosis
			Lab results										Clinical Manifestations					
			PRNT 50% Titer				IgM ELISA		Hi		RT-PCR	Virus isolation	Symptoms & sign	Haemorrhagic tendencies	Thrombo-cytopenia	Plasma leakage	Circulatory failure	
			Serotypes	Pre-illness	Acute	Conv.	Acute	Conv.	Acute	Conv.								
730	M	42	DENV-1 DENV-2 DENV-3 DENV-4	1316 140 138 13	809 76 137 18	8124 5537 >1000* 127	0.48	1.99	40	1280	Negative	Negative	Fever, headache, retro-orbital pain, myalgia, nausea, leukopenia	No	No	No	No	Dengue fever
1793	F	29	DENV-1 DENV-2 DENV-3 DENV-4	20 681 202 80	77 764 172 137	>45 000 16 750 >1000* 2126	1.3	9.3	160	2560	Negative	Negative	Fever, headache, retro-orbital pain, myalgia, sore-throat, nausea, vomiting	Tourniquet test	Yes	No	No	Dengue fever
2159	M	38	DENV-1 DENV-2 DENV-3 DENV-4	198 271 99 64	51 505 57 61	>60 000 >1000* >60 000 >40 000	0.19	1.1	80	5120	DENV-3	Negative	Fever, headache, retro-orbital pain, myalgia, sore-throat, nausea	No	No	No	No	Dengue fever
2119	F	30	DENV-1 DENV-2 DENV-3 DENV-4	75 528 36 35	<10 345 <10 <10	10 720 >1000* 12 103 4146	0.4	0.8	80	5120	DENV-3	DENV-3	Fever, headache, myalgia, nausea, vomiting	Tourniquet test	Yes	No	No	Dengue fever

*No plaques were detected even in the highest dilution (1:1000)

The second example of consecutive dengue infection was observed in a 29-year-old female. In this instance, analysis of neutralizing antibody activity two months prior to the second infection revealed a $PRNT_{50}$ titer of 359 against DENV-2. The infecting serotype was later confirmed as DENV-3 by RT-PCR. Eighteen months after the second infection, a third infection with DENV-1 was suspected based on the development of nAbs. Only the second DENV-3 infection resulted in the hospitalization of the subject. Non-specific symptoms such as fever, headache, retro-orbital pain, myalgia, sore throat, nausea and vomiting were recorded. The study volunteer also exhibited a positive tourniquet test, haemorrhagic tendencies and thrombocytopenia. Since there was neither plasma leakage nor circulatory failure the final diagnosis was DF (Table 1). The third infection with DENV-1 was milder and consequently no hospitalization was required as the patient was managed in the outpatient clinic.

A third subject, a 38-year-old male had neutralizing antibodies to DENV-2 ($PRNT_{50}$ titer: 215) a month prior to his second infection with DENV-4 (confirmed by RT-PCR, virus isolation and neutralizing antibody profile). Eighteen months later, this individual experienced a DENV-3 infection as identified by RT-PCR. Both the DENV-4 and DENV-3 infections resulted in a clinically mild disease (DF). The symptoms reported were fever, headache, retro-orbital pain, myalgia, nausea and sore throat (Tables 1 and 2).

The fourth study volunteer, a 30-year-old female, demonstrated neutralizing antibodies against DENV-2 and DENV-3 and a lower titer against DENV-4 two months prior to a DENV-4 infection that was documented by the recovery of the virus from the acute serum sample. Eleven months later, the volunteer was infected by a DENV-3 virus as evidenced by RT-PCR and virus isolation. The neutralizing antibody profile in these two episodes was in accordance with the infecting serotypes. Clinical symptoms, haemorrhagic tendencies and thrombocytopenia were reported in both episodes; however, plasma leakage, as noted by pleural effusion, ascites and hypoproteinemia, was only detected after the second infection. In this patient, the second infection was diagnosed as DHF grade II and the third as DF (Tables 1 and 2).

Previously, sequential dengue infections were recorded in 1971 in an individual from India.[5] In this instance, the first infection was in 1960 by a DENV-4 virus, followed by DENV-1 one year later and DENV-2 in 1969. All the infections manifested as a mild disease without haemorrhagic tendencies. However, dengue immune status prior to each episode of illness was unknown. Furthermore, the interval between the second and third infections was very long (8 years), which may suggest that cross-immunity to DENV-2 had disappeared.

Heterologous secondary dengue infection is often associated with more severe manifestations (DHF including dengue shock syndrome-DSS). In our case series, one of the four secondary infections resulted in DHF grade II. The enrollee was infected by DENV-4 despite having low-level neutralizing antibody to this serotype ($PRNT_{50}$ titer 29). In the other three cases, pre-illness neutralizing antibodies to the infecting serotypes were not detected. All tertiary dengue infections resulted in milder diseases. Pre-illness neutralizing antibodies to the infecting serotypes ranged from 36 to 99.

Pre-illness, acute and convalescent PRNT data from episodes with confirmed infecting serotypes (by RT-PCR or virus isolation) also showed that the highest neutralizing titers among convalescent sera may indicate that the previous infecting serotypes and the current infecting serotypes resulted in the second highest neutralizing titers in the convalescent sera. Our work supports the original antigenic sin theory first proposed by Halstead.[6]

These data support early studies that imply that the presence of neutralizing antibodies against additional dengue viruses clearly does not protect against subsequent dengue infections.

Acknowledgment

The authors thank Chairin Ma'roef, Erlin Listiyaningsih, Dra. Susana Widjaja and Dr Ardini Raksanagara for their assistance in virus isolation, RT-PCR, serology assays and clinical data collection. We are grateful to Dr Timothy H. Burgess for critical review. The opinions offered within represent those of the authors and do not reflect the official policy of the US Department of Defense or the Department of the Navy.

References

[1] George R. Dengue haemorrhagic fever in Malaysia: a review. Southeast Asian J Trop Med Public Health 1987 Sep;18(3):278-83.

[2] Suroso T. Perkembangan Kebijaksanaan Upaya Pemberantasan Penyakit Demam Berdarah Dengue (DBD) di Indonesia (1968-1996). *Presentation*. Workshop on Emerging Infectious Disease in Sawangan, East Java; June 1997.

[3] Porter KR, Beckett CG, Kosasih H, Tan RI, Alisjahbana B, Rudiman PI, Widjaja S, Listiyaningsih E, Ma'Roef CN, McArdle JL, Parwati I, Sudjana P, Jusuf H, Yuwono D, Wuryadi S. Epidemiology of dengue and dengue hemorrhagic fever in a cohort of adults living in Bandung, West Java, Indonesia. Am J Trop Med Hyg 2005 Jan;72(1):60-6.

[4] World Health Organization. Prevention and control of dengue and dengue haemorhagic fever: comprehensive guidelines. New Delhi: Regional Office for South-East Asia, 1999. SEARO Regional Publication No. 29.

[5] Myers RM, Varkey MJ. A note on sequential dengue infection, presumptive and proved, with report of an instance of a third proved attack in one individual. Indian J Med Res 1971 Aug;59(8):1231-6.

[6] Halstead SB, Rojanasuphot S, Sangkawibha N. Original antigenic sin in dengue. Am J Trop Med Hyg 1983 Jan;32(1):154-6.