

Elevated Levels of Total and Dengue Virus-Specific Immunoglobulin E in Patients With Varying Disease Severity

Penelopie Koraka,¹ Bernadette Murgue,² Xavier Deparis,³ Tatty E. Setiati,⁴ Catarina Suharti,⁴ Eric C.M. van Gorp,⁵ C.E. Hack,⁶ Albert D.M.E Osterhaus,^{1*} and Jan Groen¹

¹Laboratory for Exotic Viral Infections, Institute of Virology, Erasmus Medical Centre Rotterdam, The Netherlands

²CNR des Arbovirus et des Fieures hemorrhagiques Virales, Institut Pasteur, Paris, France

³Hôpital d'Instruction des Armees Legouest, Metz, France

⁴Department of Internal Medicine and Pediatric Department University of Diponegoro, Semarang, Indonesia

⁵Department of Internal Medicine, Slotervaart Hospital, Amsterdam, The Netherlands

⁶Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Department Clinical Chemistry, Academic Hospital Vrije Universiteit, Amsterdam, The Netherlands

The kinetics of total and dengue virus-specific immunoglobulin E (IgE) were studied in serial serum samples obtained from 168 patients, 41 of whom suffered from primary dengue virus infection and 127 suffered from secondary dengue virus infection. Seventy-one patients were classified as dengue fever, 30 as dengue hemorrhagic fever, and 67 as dengue shock syndrome. A control group included single serum samples from patients with a herpes virus infection ($n = 14$), non-dengue febrile patients ($n = 10$), and healthy blood donors ($n = 10$). Patients with dengue virus infection had higher levels of total and dengue virus-specific IgE than non-dengue patients ($P < 0.05$). Patients with secondary dengue virus infections had not significantly increased levels of both total and dengue virus-specific IgE in the acute phase of disease compared to patients with primary dengue virus infections. Dengue virus-specific IgE was significantly higher in dengue hemorrhagic fever and/or dengue shock syndrome patients compared to dengue fever and non-dengue patients ($P < 0.05$). In conclusion, this study showed elevated total and dengue virus-specific IgE serum antibody levels in the acute stage of disease. Therefore, measurement of both total and dengue virus-specific IgE serum antibodies can be used as an additional prognostic marker in the development of severe complications in dengue virus infections. In addition, the presence and increase of dengue virus-specific IgE serum antibodies in patients with dengue virus infections is suggestive of the pathogenetic role that IgE may play in the hemostatic disorders observed in dengue hemorrhagic fever and dengue shock syndrome. **J. Med. Virol.** 70:91–98, 2003. © 2003 Wiley-Liss, Inc.

KEY WORDS: anti-dengue IgE antibodies; dengue fever; dengue hemorrhagic fever

INTRODUCTION

Dengue virus, a member of the family Flaviviridae, is a mosquito-borne virus distributed mainly in the tropical and sub-tropical regions of the world [World Health Organization, 1999]. So far, four serotypes (Dengue virus 1 to 4) have been identified that cause disease in man. The virus is transmitted to humans by the bite of infected mosquitoes that are mainly of the *Aedes aegypti* and *Aedes albopictus* species. The majority of dengue virus patients will develop dengue fever characterized by fever, chills, frontal headache, myalgia, arthralgia, and a rash. In some cases, the infection may lead to more severe dengue hemorrhagic fever with plasma leakage. Usually conjunctival effusion, facial flushing, and truncal erythema also are present [World Health Organization, 1997]. According to WHO criteria, dengue hemorrhagic fever is subdivided further into four grades of severity. Grades 1 and 2 correspond to dengue hemorrhagic fever, whereas grades 3 and 4 correspond to dengue shock syndrome that is characterized by circulatory failure and might become life threatening because of profound hypovolemic shock [World Health

*Correspondence to: Prof. A.D.M.E. Osterhaus, Laboratory for Exotic Viral Infections, Department of Virology, Erasmus Medical Centre, Dr. Molenwaterplein 40 3015GD Rotterdam, the Netherlands. E-mail: A.Osterhaus@erasmusmc.ne

Accepted 9 December 2002

DOI 10.1002/jmv.10358

Published online in Wiley InterScience
(www.interscience.wiley.com)

Organization, 1997]. However, other severe forms of dengue virus have been reported that do not fit the WHO classification [Murgue et al., 1999; Harris et al., 2000].

The disease severity of dengue virus has been considered so far to be related to antibody-dependent enhancement mechanisms as a result of sequential infection with different dengue virus serotypes [Kurane and Ennis, 1997; Rothman, 1997]. There is increasing evidence that other mechanisms are also involved in the disease severity of dengue virus infections such as cytokines, viral load, and virulence of certain viral strains [Bielefeldt-Ohmann, 1997; Murgue et al., 1997; Deparis et al., 1998; Holmes and Burch, 2000].

Recently, it was demonstrated that IgM, IgG 1, and IgG 3 antibodies were the predominant immunoglobulins through the course of illness in dengue fever, dengue hemorrhagic fever, and dengue shock syndrome patients. Increased levels of dengue-specific IgA, IgG 1, and IgG 4 serum antibodies are risk markers for the development of dengue hemorrhagic fever/dengue shock syndrome [Koraka et al., 2001]. High levels of total and virus-specific IgE are associated with an anaphylactic reaction [Bochner and Lichtenstein, 1991]. In patients with hemorrhagic fever virus infections, it was shown that both total and specific IgE levels are increased [Pavri and Prasad, 1980; Pavri et al., 1980; Pavri, 1989; Alexeyev et al., 1994]. Recently, it was demonstrated that the total IgE levels in patients with a history of dengue virus infection increased when compared to individuals without a history of dengue virus infection [Miguez-Burbano et al., 1999]. This relationship between elevated total IgE and dengue virus infection may indicate the role of IgE in immunohomeostasis. Increased levels of total IgE in dengue virus infected patients may also indicate that a balanced immune response might lead to disease resolution, rather than the induction of a particular T-helper cell pathway. It is also known that infections with dengue virus induce interleukin 4 (IL-4) production [Chaturvedi et al., 2000], which is involved in IgE synthesis in humans [Gascan et al., 1991].

The aim of the present study was to investigate whether there is a correlation between total and dengue

virus-specific IgE response in relation to disease severity and if dengue virus-specific IgE antibodies could be used as a prognostic marker for disease severity.

MATERIALS AND METHODS

Serum Samples

Serial serum samples were collected from 168 patients with clinically and serologically confirmed dengue virus infection. According to the criteria of the World Health Organization [World Health Organization, 1997], 71 patients from the 168 dengue virus infected patients were defined as dengue fever cases, 30 patients were defined as dengue hemorrhagic fever cases, and the remaining 67 patients were defined as dengue shock syndrome cases. Samples had been collected during the 1995–1996 dengue virus epidemic in Indonesia, where all dengue virus serotypes were circulating with Dengue virus 3 being the most predominant serotype [Graham et al., 1999]. The age of the patients varied between 7 months and 14 years and 52% of the patients were females. The characteristics of the dengue virus infected patients are summarized in Table I. A control group included fourteen samples obtained from patients with herpes virus infection (seven patients with an Epstein-Barr virus infection and seven patients with cytomegalovirus infection), ten samples obtained from patients with a febrile illness other than dengue virus, who were all residents of the same endemic areas in Indonesia as the dengue virus infected individuals, and ten samples from Dutch healthy blood donors. In addition, eight samples were included from patients with elevated levels of IgE to serve as positive controls for the measurement of total IgE.

Serology

IgM/IgG enzyme immunoassay .Dengue virus-specific IgM and IgG antibodies were measured in the serum samples of the patients using a commercially available EIA kit (Focus Technologies, Cypress CA), and carried out according to the procedures described by the manufacturer.

TABLE I. Classification and Characteristics of Dengue Virus Infected Patients Included in the Study

Classification ^a	Patients (n)	Sex		Mean age (years)	Mean IgM/IgG (ratio)
		Male	Female		
Primary infections					
DF	29	13	16	7.0	9.4
DHF	5	4	1	6.8	9.7
DSS	7	3	4	6.2	1.4
Subtotal	41	20	21	6.7	6.8
Secondary infections					
DF	42	24	18	8.7	0.3
DHF	25	11	14	8.7	0.4
DSS	60	25	35	7.1	0.3
Subtotal	127	60	67	8.2	0.3

^aDF, dengue fever; DHF, dengue hemorrhagic fever; DSS, dengue shock syndrome.

Total IgE enzyme immunoassay .Medium binding plates (Greiner, Frick Kenhausen, Germany) were coated with goat anti-human IgE antibodies (Cappel Cooper Biomedical, West-Chester, PA), diluted 1:2,000 in carbonate buffer (pH 9.6) overnight at 4°C. After blocking the plates with 5% (w/v) skim milk (ELK Campina, Eindhoven, The Netherlands) in PBS for 30 min at 37°C, 100 µl of diluted serum samples were added to the plates. Serum samples were diluted 1:100 in PBS containing 0.2% (w/v) milk, 0.1% (w/v) bovine serum albumin (Sigma Chemical Co., St. Louis, MO), 0.1% (w/v) ovalbumin (Sigma Chemical Co., St. Louis, MO), 1% (v/v) fetal bovine serum (ICN Biochemicals, Inc., OH), and 5% (v/v) normal goat serum (ICN Biochemicals, Inc., OH) (EIA-buffer). After 1 hour incubation at 37°C, unbound antibodies were washed away and 100 µl of rabbit anti-human IgE peroxidase-labeled conjugate (Dako, Glostrup, Denmark), diluted 1:500 in EIA-buffer, was added to the plates. Plates were incubated for an additional hour at 37°C and excess of conjugate was washed away. The plates were developed with 2,2,4,4 tetramethylbenzidine for 15 minutes at room temperature, the reaction was stopped with the addition of 1 N H₂SO₄ and the extinction of the samples was read at 450 nm (with 620 nm reference filter).

Dengue virus-specific IgE enzyme immunoassay. Plates were coated and blocked as described for the total IgE enzyme immunoassay. Serum samples were diluted 1:100 in EIA-buffer and added in 100 µl volumes on the plates for 1 hour at 37°C. Unbound antibodies were washed away and a mixture of all four dengue virus serotypes prepared as previously described [Koraka et al., 2001] was added to the plates diluted 1:20 in EIA-buffer supplemented with 5% (v/v) normal human serum. After overnight incubation at 4°C, plates were washed to remove excess of antigen and incubated for 1 hour at 37°C with an anti-Flavivirus monoclonal antibody, peroxidase conjugated (Focus Technologies, Cypress, CA) diluted 1:2,000 in EIA-buffer with normal human serum. Plates were washed three times and developed as described in the procedure for total IgE.

Calculations

The cut-off serum of the manufacturer was used for the calculation of IgM and IgG antibody ratios.

Dengue virus infections were defined as primary when the ratio of dengue virus-specific IgM/IgG serum antibodies was higher than one; if the ratio of dengue virus-specific IgM/IgG serum antibodies was lower than one, the infections were defined as secondary dengue virus cases [World Health Organization, 1997].

A standard curve was constructed using different dilutions of a positive reference serum sample for the calculation of total IgE levels in the serum samples. The concentration of the total IgE antibodies was calculated after adjustment of the dilution factor.

The ratios of the dengue virus-specific IgE antibodies was calculated according to the following formula: Dengue virus-specific IgE ratio = (O.D. sample – O.D.

blank)/(mean of negative controls + 3 × S.D), where O.D. is the optical density (extinction) of each sample.

Univariate and multivariate statistical analysis were undertaken successively by using BMDP software. The univariate statistical tests allow the showing of a relation between two variables but without adjusting for the influence of other variables. By carrying out a multivariate statistical analysis, after adjusting for the other variables and their interactions, it could be possible that a significant relation using a univariate test disappears. When the variables are numerous, the results of the univariate analysis may be useful in order to select the variables to be included in the multivariate analysis.

First, univariate analysis was carried out. Using the Spearman test, Student test, and ANOVA, which allow the assessing of the significance of group differences of a grouping variable, the relation of the dependent variables, i.e., the level of dengue virus-specific IgE, and the independent variables, i.e. age, sex, and days post onset of illness of the patients, the severity of infection, immunity to dengue virus (primary and multiple dengue virus infection), and the level of total IgE, IgM, and IgG were assessed.

Second, a multivariate analysis was carried out by using analysis of covariance (ANCOVA). Since the distribution of the data was normal as determined by computer analysis and in order to take into account the interactions of the independent variable in a global model, with a second analysis, the independent variables were included in an ANCOVA descriptive model. An analysis of covariance can be viewed as a combination of analysis of variance (ANOVA) and regression. This was used to refine the univariate analysis by adjusting for covariates that may be related linearly to the outcome that is being investigated. Consequently, ANCOVA is conducted in order to show some variables, which could be significant using the univariate analysis and which have finally no significant role. A two-sided *P*-value <0.05 was considered to represent a significant difference. The results of the ANCOVA model allow assessment of the real influence of the studied independent variable on the IgE-specific level because this model takes into account the interactions of the different variables.

RESULTS

Classification of Dengue Virus Infected Patients

The clinical classification and the characteristics of the patients included in this study are summarized in Table I. Based on the IgM/IgG ratio of the serum sample collected at the acute phase of illness, 41 out of the 168 dengue virus infected patients had a primary dengue virus infection (mean IgM/IgG ratio, 6.8) and 127 patients had a secondary dengue virus infection (mean IgM/IgG ratio, 0.3). Of the 41 primary infections, 29 patients had dengue fever (mean IgM/IgG ratio, 9.4), five patients developed dengue hemorrhagic fever

(mean IgM/IgG ratio, 9.7), and seven patients developed dengue shock syndrome (mean IgM/IgG ratio, 1.4). Of the 127 secondary infections, 42 patients had dengue fever (mean IgM/IgG ratio, 0.3), 25 patients developed dengue hemorrhagic fever (mean IgM/IgG ratio, 0.4), and 60 patients developed dengue shock syndrome (mean IgM/IgG ratio, 0.3) (Table I).

Kinetics of Total and Dengue Virus-Specific IgE Serum Antibodies in Dengue Virus Infected Patients

Levels of total IgE remained stable during acute dengue fever (days 1 to 7 after onset of symptoms), early convalescent dengue fever (days 8 to 13 after onset of symptoms), and the convalescent phase of dengue fever (more than 14 days after onset of symptoms) with mean $\pm$ standard deviation varying between 125 ± 196 , 108 ± 162 , and 124 ± 203 U/L in the respective phases of illness (Fig. 1a). In contrast, dengue virus-specific IgE serum antibodies increased from a mean ratio of $\pm$ SD 0.9 ± 1.1 in acute dengue fever to 1.9 ± 1.6 and 1.5 ± 1.4 during early convalescent and convalescent dengue fever, respectively (Fig. 1b).

Elevated levels of total IgE serum antibodies were also observed in the dengue hemorrhagic fever group of patients with a mean $\pm$ SD U/L 154 ± 206 , 50 ± 84 , and 106 ± 133 during acute, early convalescent and convalescent dengue hemorrhagic fever respectively (Fig. 1a). Dengue virus-specific IgE serum antibodies in dengue hemorrhagic fever patients were elevated at mean ratios 2.2 ± 1.6 , 2.0 ± 1.3 , and 1.8 ± 1.1 during

acute, early convalescent, and convalescent phase of dengue hemorrhagic fever, respectively (Fig. 1b).

Patients that developed dengue shock syndrome had the highest mean values of total IgE serum antibodies in the acute phase of infection (331 ± 285 U/L) and during the early convalescent phase of infection (300 ± 265 U/L), although the levels of total IgE decreased to 56 ± 86 U/L in convalescence (Fig. 1a). Dengue virus-specific IgE serum antibodies in dengue shock syndrome patients were at rather stable levels during all stages of infection with mean $\pm$ SD ratios of 2.6 ± 1.7 , 3.3 ± 2.1 , and 2.7 ± 2.3 during acute, early convalescent, and convalescent phase of infection, respectively (Fig. 1b).

Levels of Total and Dengue Virus-Specific IgE Serum Antibodies in Acute Phase Dengue Virus Infected Patients

Results of the univariate analysis. Dengue virus-specific IgE serum antibodies were significantly related to the day after the onset of symptoms, severity of disease, immunity to dengue virus (primary vs. secondary infections) and the level of total IgE and IgG. Dengue virus-specific IgE serum antibodies were not significantly related to the age of the patients or to the levels of IgM antibodies (Tables II–IV). These results have to be confirmed by the results of the multivariate analysis.

Results of the ANCOVA After Adjusting for Covariates

Taking into account the influence of the other independent variables (Table V), the ANCOVA showed that

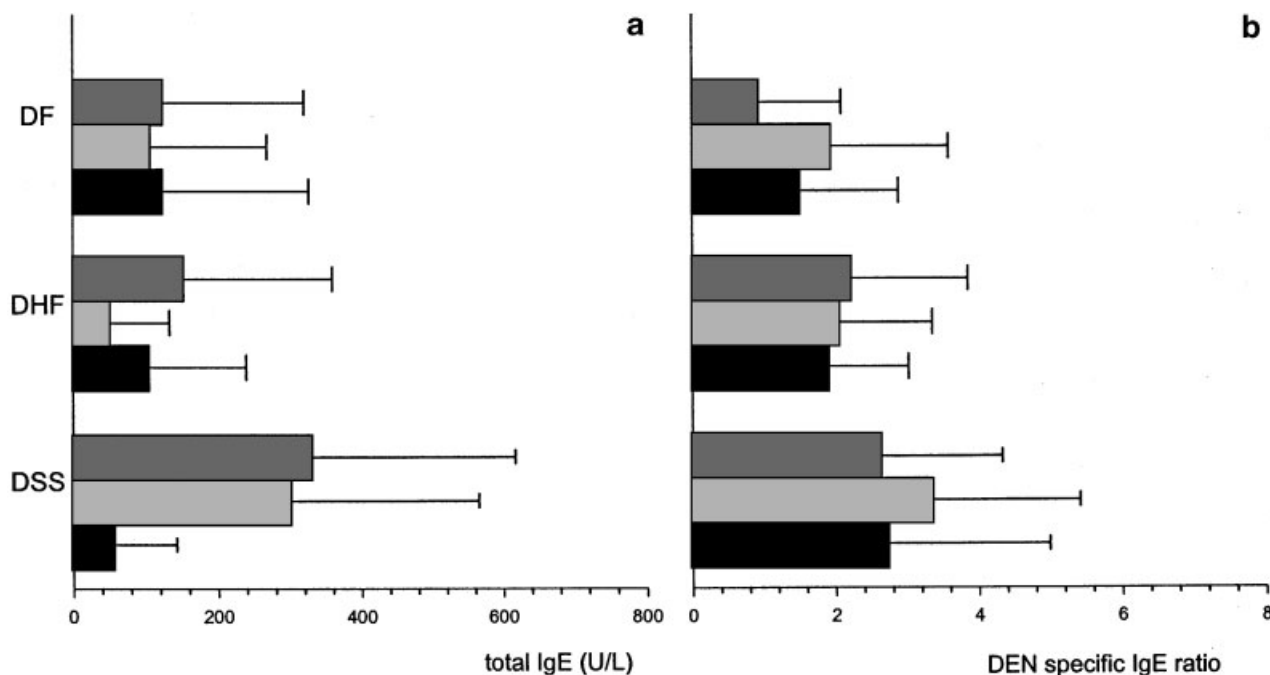


Fig. 1. Kinetics of total and dengue virus (DEN)-specific IgE serum antibodies among the respective patient groups [dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS)]. Dark gray bars represent the acute phase of disease, light gray bars represent early convalescent phase, and black bars represent convalescent phase of the disease.

TABLE II. Relation of the Levels of Dengue Virus (DEN)-Specific IgE Antibodies and the Day After Onset of Symptoms and the Levels of Total IgE and Dengue Virus-Specific IgM and IgG

	<i>P</i> value (Pearson test)
Day post onset of symptoms	0.0011
Level of total IgE	$<10^{-9}$
Level of DEN-specific IgM	0.53
Level of DEN-specific IgG	$<10^{-17}$

the level of dengue virus-specific IgE increased significantly over time (regression coefficient = 0.04, $P = 0.027$). The level of dengue virus-specific IgE increased significantly with the level of IgG (regression coefficient = 0.16, $P = 0.0001$) and to a lesser degree with the level of total IgE (regression coefficient = 0.001, $P = 0.0006$). Dengue virus-specific IgE decreased slowly but significantly with the age of the patient (regression coefficient = -0.06, $P = 0.016$). The level of IgM had no influence on the level of dengue virus-specific IgE. The model also showed no influence of the immunological status of the dengue virus infections ($P = 0.19$) and no significant interaction between the immunological status and the severity of dengue illness ($P = 0.38$) (Table V).

Taking into consideration the other independent variables, the ANCOVA allowed us to calculate adjusted values of the dengue virus-specific IgE serum antibodies and it was demonstrated that the level of dengue virus-specific IgE was higher among dengue shock syndrome patients (Fig. 2). In detail, the level of dengue virus-specific IgE serum antibodies increased from a mean ratio of 1.60 in primary dengue fever patients to a mean ratio 2.03 and 1.24 in primary dengue hemorrhagic fever 1 and 2 patients, respectively (dengue hemorrhagic fever patients), to reach the highest levels in primary dengue shock syndrome patients (mean ratios 1.85 and 2.85 in dengue hemorrhagic fever grade 3 and 4 patients, respectively). The trend of dengue virus-specific IgE serum antibodies during secondary infections was not significantly different with primary infections (Table V), increasing from a mean ratio of 1.37 in dengue fever patients to mean ratios of 2.36 and 1.82 in dengue

hemorrhagic fever patients (dengue hemorrhagic fever 1 and 2, respectively) and reached the highest values in dengue shock syndrome patients with mean ratios of 2.35 and 3.72 among dengue hemorrhagic fever 3 and 4 patients, respectively.

Comparison of Total and Dengue Virus-Specific IgE Serum Antibodies Among Dengue and Non-Dengue Febrile Patients

Although the levels of total IgE serum antibodies between reference IgE serum (positive controls) and dengue fever, dengue hemorrhagic fever, and dengue shock syndrome patients were not significantly different, the levels of dengue virus-specific IgE serum antibodies were significantly different between IgE reference serum and dengue hemorrhagic fever patients ($P = 0.03$) or between IgE reference serum and dengue shock syndrome patients ($P = 0.002$). Statistical analysis between the control group (non-dengue febrile patients and blood donors) and acute phase dengue fever patients did not revealed a significant difference between these two groups ($P = 0.8$ and $P = 0.4$ for total and dengue virus-specific IgE, respectively). The levels of total IgE antibodies did not differ significantly between non-dengue patients and acute phase dengue hemorrhagic fever patients ($P = 0.539$); however, dengue virus-specific IgE antibodies were significantly different between acute phase dengue hemorrhagic fever patients and non-dengue patients ($P = 0.000$). In acute phase dengue shock syndrome patients, the levels of total and dengue virus-specific IgE serum antibodies were significantly higher than in non-dengue patients ($P = 0.000$ for both total and dengue virus-specific IgE serum antibodies) (Fig. 3).

DISCUSSION

In the present study, the levels of total and dengue virus-specific IgE serum antibodies were measured in patients with dengue virus infection of varying disease severity. We showed increased levels of total and dengue virus-specific IgE serum antibodies in the acute phase of illness in patients with primary and secondary dengue virus infections. The levels of total IgE serum antibodies

TABLE III. Comparison of the Level of Dengue Virus-Specific IgE Serum Antibodies Following the Level of Disease Severity and the Age Category of the Patients

	Mean	Standard deviation	df ^a	F value	<i>P</i> value (ANOVA)
Dengue severity (WHO classification)			4	28.6	$<10^{-21}$
DF	1.06	1.27			
DHF 1	2.37	1.52			
DHF 2	1.89	1.47			
DHF 3	2.66	1.6			
DHF 4	3.78	2.89			
Age			2	1.13	0.32
0–5 years	2.27	2.17			
6–10 years	2.05	1.74			
11–15 years	1.89	1.58			

^aDegrees of freedom.

TABLE IV. Comparisons of the Mean Level of Dengue Virus (DEN)-Specific IgE Following the Sex and the Immunity Status of the Patient

	Mean	Standard deviation	<i>P</i> value (Student's test)
Sex			
Male	1.89	1.73	0.08
Female	2.19	1.82	
Immunity status			
Primary DEN infection	1.00	1.32	<10 ⁻⁹
Secondary DEN infection	2.34	1.79	

in primary and secondary dengue virus infected patients measured in this study are in agreement with the results studied in similar patient groups, by Miguez-Burbano et al. [1999]. The findings that total IgE serum antibodies are elevated in patients with primary and secondary dengue virus infections compared to non-dengue patients indicates that dengue virus infections exert a strong stimulatory effect on IgE synthesis. The non-dengue group of patients included patients with acute Epstein Barr virus and cytomegalovirus infections, which also cause elevation of the levels of total IgE in the serum [Bahna et al., 1984; van Loon et al., 1987], resulting in a non-significant difference between the non-dengue patients and patients with a primary dengue virus infection. Increased levels of total IgE serum antibodies in the acute phase of disease of both primary and secondary infections may indicate that dengue virus infections also stimulate a non-antigen-specific activation pathway for IgE production.

It was also demonstrated that patients with dengue shock syndrome had significantly elevated levels of total IgE serum antibodies as compared to patients with dengue fever ($P = 0.000$) or dengue hemorrhagic fever ($P = 0.002$). In addition, all dengue virus infected patients had elevated levels of total IgE (when compared with total positive IgE reference samples); however patients with dengue virus infection had significantly higher levels of total IgE serum antibodies than patients with a non-dengue infection ($P < 0.05$). Previous reports have suggested that elevated levels of total IgE serum antibodies in patients that suffer from hemorrhagic fever virus infections are associated with disease severity [Pavri and Prasad, 1980; Miguez-Burbano et al., 1999]. Pavri and colleagues have suggested that

increased levels of total IgE serum antibodies are implicated in the pathogenesis of Kyasanur Forest disease, Japanese B encephalitis and dengue hemorrhagic fever [Pavri and Prasad, 1980; Pavri et al., 1980; Pavri, 1989]. Alexeyev et al. [1994] demonstrated elevated levels of total and Puumala virus-specific IgE serum antibodies early in the course of hemorrhagic fever with renal syndrome, suggesting that IgE may be involved in the pathogenesis of this disease.

Furthermore, the levels of dengue virus-specific IgE serum antibodies in patients with dengue virus infection were measured. The results showed that dengue virus-specific IgE was significantly elevated in dengue hemorrhagic fever and dengue shock syndrome patients as compared to non-dengue or dengue fever patients ($P < 0.05$). In addition, all non-dengue patients and IgE positive reference serum samples were negative when tested for the presence of dengue virus-specific IgE serum antibodies, resulting in high specificity of the dengue virus-specific IgE serum antibody measurement assay. It was also demonstrated that dengue virus-specific IgE serum antibodies in the acute phase of dengue virus infection is significantly higher in patients that developed dengue hemorrhagic fever and/or dengue shock syndrome compared to dengue fever patients ($P < 0.001$). In addition, the levels of dengue virus-specific IgE serum antibodies were significantly associated with the day after onset of symptoms of the patients as well as levels of total IgE and IgG. In contrast, the levels of dengue virus-specific IgE serum antibodies did not correlate with the age or the sex of the patients or the level of IgM serum antibodies or the immunological status. These findings suggest that these parameters could be used as prognostic markers for

TABLE V. Level of Significance of the Independent Variables Included in the Descriptive ANCOVA Model

Source of ANCOVA	Sum of squares	Regression coefficient ^a	df ^b	F value	<i>P</i> value
Severity of DEN	53.7		4	6.36	0.0001
Immunity status	3.6		1	1.73	0.19
Interaction: severity and immunity status	8.9		4	2.22	0.38
Sex	0.79		1	0.38	0.53
Day post onset of symptoms	10.38	0.04	1	4.92	0.028
Level of total IgE	25.6	0.001	1	12.14	0.0006
Level of IgM	0.005	-0.0006	1	0	0.96
Level of IgG	58.64	0.16	1	27.81	<10 ⁻⁴
Age	12.41	-0.06	1	5.89	0.016

^aOnly available for the quantitative independent variables, i.e., the covariables.

^bDegrees of freedom.

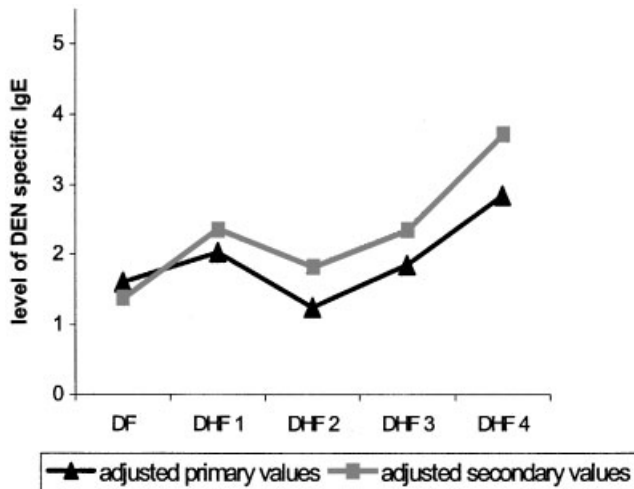


Fig. 2. Levels of dengue virus (DEN)-specific IgE in correlation with dengue disease severity. The adjusted values resulted from the ANCOVA model that took into account the influence of all variables in the model.

developing severe complications during infection with dengue virus.

The implication of IgE in increased vascular permeability (a common clinical complication in severe dengue virus infection) has been reported in the past [Boesiger et al., 1998]. Proinflammatory cytokines, such as IL-1 β and tumor necrosis factor, triggered by IgE may contribute to this phenomenon [Borish et al., 1991]. Both of these cytokines are produced during dengue virus

infection [Chaturvedi et al., 2000]. An immunoglobulin switch to the IgE class is stimulated by Th2 cytokines, namely, IL-4 and IL-13 to a lower extent [Punnonen et al., 1993; Carballido et al., 1995]. Considering that Th2 immune responses are correlated with severe pathology in several other viral infections [Mosmann and Sad, 1996] and that in dengue shock syndrome, a shift from Th1 to Th2 responses is observed [Chaturvedi et al., 2000], we might speculate that elevated IgE serum antibodies may be associated with severity of dengue virus disease. Recently, we demonstrated that dengue virus infected patients that developed dengue shock syndrome had increased levels of dengue virus-specific IgG 4 serum antibodies compared to patients with dengue fever/dengue hemorrhagic fever [Koraka et al., 2001]. Production of IgG 4 is also stimulated by IL-4 as with the production of IgE serum antibodies. In addition, dengue virus-specific IgE serum antibodies may play a role in hemostatic disorders seen in dengue hemorrhagic fever, via induction and release of vasoactive factors such as histamine, which has been shown to be increased in dengue hemorrhagic fever patients [Srichaikul and Nimmannitya, 2000].

In conclusion, the pre-existing levels of total and dengue virus-specific IgE serum antibodies in patients with dengue virus infections, and in particular the significantly higher levels of these antibodies in the acute phase of dengue hemorrhagic fever/dengue shock syndrome, strongly suggest that IgE responses may be involved in the pathogenesis of disease or be a reflection of the underlying immunological pathogenetic

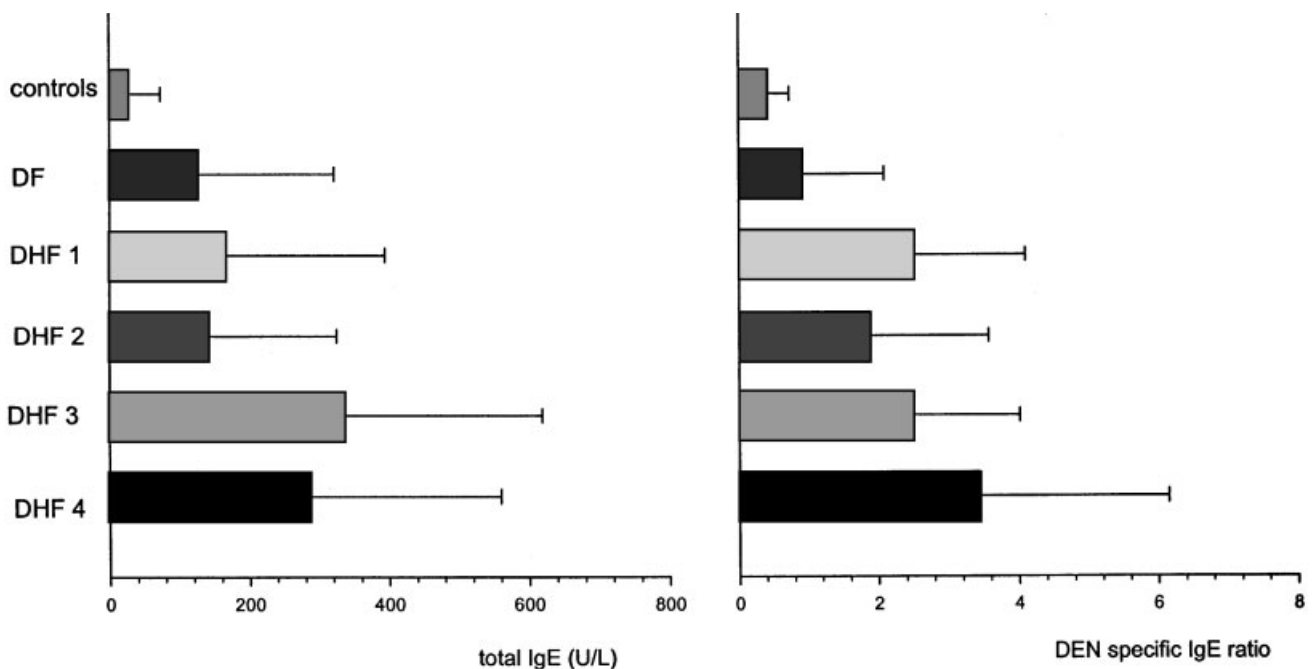


Fig. 3. Levels of total and dengue virus (DEN)-specific IgE serum antibodies among the respective patient groups and controls in the acute phase of illness. DHF 1 and 2 patients compose the dengue hemorrhagic fever group of patients. DHF 3 and 4 patients compose the dengue shock syndrome group of patients. The control group includes patients with EBV and CMV infection, non-dengue febrile patients and patients with elevated levels of total IgE (positive controls).

mechanism. Moreover, the results of this study suggest that elevated levels of total and dengue virus-specific IgE serum antibodies in the acute phase of dengue virus infection may be prognostic for developing severe complication of disease.

REFERENCES

- Alexeyev OA, Ahlm C, Billheden J, Settergren B, Wadell G, Juto P. 1994. Elevated levels of total and Puumala virus-specific immunoglobulin E in the Scandinavian type of hemorrhagic fever with renal syndrome. *Clin Diagn Lab Immunol* 1:269–272.
- Bahna SL, Heiner DC, Horwitz CA. 1984. Sequential changes of the five immunoglobulin classes and other responses in infectious mononucleosis. *Int Arch Allergy Appl Immunol* 74:1–8.
- Bielefeldt-Ohmann H. 1997. Pathogenesis of dengue virus diseases: missing pieces in the jigsaw. *Trends Microbiol* 5:409–413.
- Bochner BS, Lichtenstein LM. 1991. Anaphylaxis. *N Engl J Med* 324:1785–1790.
- Boesiger J, Tsai M, Maurer M, Yamaguchi M, Brown LF, Claffey KP, Dvorak HF, Galli SJ. 1998. Mast cells can secrete vascular permeability factor/vascular endothelial cell growth factor and exhibit enhanced release after immunoglobulin E-dependent upregulation of ϵ epsilon receptor I expression. *J Exp Med* 188:1135–1145.
- Borish L, Mascali JJ, Rosenwasser LJ. 1991. IgE-dependent cytokine production by human peripheral blood mononuclear phagocytes. *J Immunol* 146:63–67.
- Carballido JM, Schols D, Namikawa R, Zurawski S, Zurawski G, Roncarolo MG, de Vries JE. 1995. IL-4 induces human B cell maturation and IgE synthesis in SCID-hu mice: inhibition of ongoing IgE production by in vivo treatment with an IL-4/IL-13 receptor antagonist. *J Immunol* 155:4162–4170.
- Chaturvedi UC, Agarwal R, Elbishbishi EA, Mustafa AS. 2000. Cytokine cascade in dengue hemorrhagic fever: implications for pathogenesis. *FEMS Immunol Med Microbiol* 28:183–188.
- Deparis X, Murgue B, Roche C, Cassar O, Chungue E. 1998. Changing clinical and biological manifestations of dengue during the dengue-2 epidemic in French Polynesia in 1996/97: description and analysis in a prospective study. *Trop Med Int Health* 3:859–865.
- Gascan H, Gauchat JF, Aversa G, Van Vlasselaer P, de Vries JE. 1991. Anti-CD40 monoclonal antibodies or CD4+ T cell clones and IL-4 induce IgG4 and IgE switching in purified human B cells via different signaling pathways. *J Immunol* 147:8–13.
- Graham RR, Juffrie M, Tan R, Hayes CG, Laksono I, Ma'roef C, Erlin, Sutaryo J, Porter KR, Halstead SB. 1999. A prospective seroepidemiologic study on dengue in children four to nine years of age in Yogyakarta, Indonesia. I. Studies in 1995–1996. *Am J Trop Med Hyg* 61:412–419.
- Harris E, Videe E, Perez L, Sandoval E, Tellez Y, Perez ML, Cuadra R, Rocha J, Idiaquez W, Alonso RE, Delgado MA, Campo LA, Acevedo F, Gonzalez A, Amador JJ, Balmaseda A. 2000. Clinical, epidemiologic, and virologic features of dengue in the 1998 epidemic in Nicaragua. *Am J Trop Med Hyg* 63:5–11.
- Holmes EC, Burch SS. 2000. The causes and consequences of genetic variation in dengue virus. *Trends Microbiol* 8:74–77.
- Koraka P, Suharti C, Setiati TE, Mairuhu AT, Van Gorp E, Hack CE, Juffrie M, Sutaryo J, Van Der Meer GM, Groen J, Osterhaus AD. 2001. Kinetics of dengue virus-specific serum immunoglobulin classes and subclasses correlate with clinical outcome of infection. *J Clin Microbiol* 39:4332–4338.
- Kurane I, Ennis FA. 1997. Immunopathogenesis of dengue virus infections. In: Gubler DJ, Kuno G, editors. *Dengue and Dengue haemorrhagic fever*. London: CAB International. p 273–290.
- Miguez-Burbano MJ, Jaramillo CA, Palmer CJ, Shor-Posner G, Velasquez LS, Lai H, Baum MK. 1999. Total immunoglobulin E levels and dengue infection on San Andres Island, Colombia. *Clin Diagn Lab Immunol* 6:624–626.
- Mosmann TR, Sad S. 1996. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 17:138–146.
- Murgue B, Cassar O, Guigon M, Chungue E. 1997. Dengue virus inhibits human hematopoietic progenitor growth in vitro. *J Infect Dis* 175:1497–1501.
- Murgue B, Deparis X, Chungue E, Cassar O, Roche C. 1999. Dengue: an evaluation of dengue severity in French Polynesia based on an analysis of 403 laboratory-confirmed cases. *Trop Med Int Health* 4:765–773.
- Pavri K. 1989. Clinical, clinicopathologic, and hematologic features of Kyasanur Forest disease. *Rev Infect Dis* 11:S854–S859.
- Pavri KM, Prasad SR. 1980. T suppressor cells: role in dengue hemorrhagic fever and dengue shock syndrome. *Rev Infect Dis* 2:142–146.
- Pavri KM, Ramamoorthy CL, Dhorje S. 1980. Immunoglobulin E in patients with Japanese encephalitis. *Infect Immunol* 28:290–291.
- Punnonen J, Aversa G, Cocks BG, McKenzie AN, Menon S, Zurawski G, de Waal MR, de Vries JE. 1993. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci USA* 90:3730–3734.
- Rothman AL. 1997. Viral pathogenesis of dengue infections. In: Gubler DJ, Kuno G, editors. *Dengue and Dengue haemorrhagic fever*. London: CAB International. p 245–272.
- Srichaikul T, Nimmannitya S. 2000. Haematology in dengue and dengue haemorrhagic fever. *Baillieres Best Pract Res Clin Haematol* 13:261–276.
- van Loon AM, Heessen FW, van der Logt JT. 1987. Antibody isotype response after human cytomegalovirus infection. *J Virol Methods* 15:101–107.
- World Health Organization. 1997. *Dengue hemorrhagic fever: diagnosis, treatment, prevention and control*. Second edition.
- World Health Organization. 1999. *Strengthening implementation of the global strategy for Dengue/Dengue Hemorrhagic Fever prevention and control*. Report of the informal consultation, October 1999.