

DENGUE 2 VIRUS ENHANCEMENT IN ASTHMATIC AND NON ASTHMATIC INDIVIDUAL

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During the 1981 dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) Cuban epidemic, bronchial asthma (BA) was frequently found as a personal or family antecedent in dengue hemorrhagic fever patients.

Considering that antibody dependent enhancement (ADE) plays an important role in the etiopathogenic mechanism of DHF/DSS, we decide to study the Dengue 2 virus (D2V) capability of replication in peripheral blood leukocytes (PBL) from asthmatic patients and healthy persons.

In 90% of asthmatic patients and 53.8% of control group it was obtained PBL with a significant D2V enhancing activity ($X^2 p < 0.01$). Power enhancement was higher in asthmatic group.

This is the first in vitro study relating BA and the dengue 2 virus immuno enhancement. The results obtained support the role of BA as a risk factor for DHF/DSS as already described on epidemiological data.

Key words: dengue – macrophages – antibody dependent – enhancement

Dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) is one of the most important causes of hospitalization and death among children in at least eight countries in southeast Asia where more than 1.5 million hospitalized patients and 33,000 deaths were reported since 1950's (Pinheiro, 1989).

Recently, there has been an increase in the incidence of dengue fever in the Americas and in Caribbean countries where the simultaneous circulation of at least three viral serotypes has been documented (Pinheiro, 1989).

At the same time there has been an increase in DHF/DSS cases and a second epidemic in the hemisphere was reported in Venezuela (Programa de Enfermedades Trans-

misibles, 1990). Previously a large outbreak of DHF/DSS occurred in Cuba in 1981 (Kouri et al., 1989). Public health authorities warn that DHF/DSS is a risk in those countries where viruses are circulating specially Brazil, Equador and Paraguay which present an epidemiological pattern similar to that observed in Cuba prior to the 1981 outbreak (Pinheiro, 1992).

Although the syndrome pathogenesis is still in study, antibody dependent enhancement (ADE) is considered by many authors the most likely mechanism of disease cause (Halstead, 1988).

The virus replication in humans appears to be restricted to mononuclear phagocytes, in which the virus replicates to higher titers in the presence than in the absence of sub-neutralizing concentrations of homotypic or heterotypic antibodies, acquired during a previous infection (Halstead, 1982; Halstead et al., 1980; Porterfield, 1982).

Bronchial asthma (BA), a condition in some extent linked to the individual genetic, is highly

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prevalent (11% in children and 7% in adults) in Cuba. Rodriguez de la Vega et al. (1983) reported in 1983 the prevalence of BA in a representative sample of the Cuban population, according to the 1981 National Census Population. In this study a questionnaire was completed and a physical examination was performed by medical doctors to all individuals included. The total sample population was 22,751 persons.

From some studies done in hospitalized and fatal cases during the 1981 dengue Cuban epidemic, BA was identified as a possible risk factor of the severe disease (Bravo et al., 1987; Guzmán et al., 1987). This is a new finding in the history of DHF/DSS.

The purpose of this research is to study if there are differences between the capability of

human peripheral blood leukocytes (PBL) from asthmatic and non-asthmatic individuals to support dengue 2 virus replication in the presence of dengue enhancing antibody.

MATERIALS AND METHODS

Case studies – PBL were obtained from 30 white patients with BA attending the National Asthmatic Outpatient Service of the Asclepio Polyclinic. They were selected during seven days in the order of arrival to the service, and tested for dengue antibodies. Those negatives were included in the study (Table I).

Eighteen of the patients had BA antecedent since childhood; 17 had ten or more episodes of acute disease yearly. Twenty four had one or more hospitalizations for acute asthmatic episodes or other complications.

TABLE I

Enhancing activity in the asthmatic patients (n = 30)

Case	Sex	Age	Previous hospitalization	Age at onset BA ^a	Average No. annual episodes	Power ^b
1	M	50	+	3	NR	3
2	M	22	+	4	30	5
3	F	46	+	22	20	7.8
4	M	49	+	39	NR	2.6
5	M	37	–	6	40	7
6	F	53	+	45	20	23
7	M	65	+	33	20	39
8	F	48	–	13	3	11
9	M	63	+	NR	10	26
10	F	36	–	34	20	23
11	M	60	+	27	20	1.9
12	M	51	+	40	20	2.6
13	M	26	–	4	30	4.9
14	M	30	+	5	20	3
15	F	51	+	3	NR	0
16	F	24	+	1	NR	7.5
17	M	49	+	4	10	2.6
18	F	62	+	NR	<10	13
19	F	47	+	5	<10	0
20	M	35	+	1	20	2.3
21	F	36	+	1	NR	4.4
22	M	45	+	5	<10	6.3
23	F	28	+	22	20	14
24	F	32	+	1	20	4.4
25	F	18	+	11	NR	0
26	F	49	–	3	<10	2.6
27	M	52	+	4	20	1.5
28	F	62	+	30	20	6
29	M	39	–	1	<10	3.1
30	F	51	+	20	<10	4

^a: bronchial asthma.

^b: enhancement power compared to yield from cultures without dengue 1 immune serum.

Control were selected among 30 healthy non allergic white individuals; 13 of them, negatives to dengue antibodies, were included in the study as a control group (Table II).

TABLE II

Enhancing activity in control group (n = 13)

Case	Sex	Age	Power ^a
1	F	28	25
2	M	41	10.3
3	M	47	2
4	F	36	9.5
5	M	46	6.8
6	F	26	0
7	F	34	5.7
8	M	33	0
9	F	33	0
10	M	33	-
11	M	29	0
12	F	24	1.4
13	F	38	0

a: enhancement power compared to yield from cultures without dengue 1 immune serum.

Virus preparation and assay – Was used Dengue 2, strain A15 (D2V), isolated from the serum of a patient who acquired dengue fever during the 1981 epidemic (Kouri et al., 1983). The virus had been propagated in the brain of newborn mice, and prepared for use as a 10% suspension (W/V) in Hank's solution with 2% calf serum and antibiotics. Virus was quantitated by a plaque assay and stored at -70 °C until use.

Leukocyte separation, infection and culture – Blood obtained from adult individuals without dengue antibodies (tested by hemagglutination-inhibition and ELISA test) was anticoagulated in preservative free heparin (20 IU/ml fluid concentration). For this study, PBL were recovered by the density flotation method (Ficoll-Hypaque) described by Boyum (1968). PBL were separated by centrifugation at 400 x g for 30 min and washed three times in PBS, suspended, counted and resuspended at 1 x 10⁶ cells/ml in RPMI 1640 medium with 20 mM hepes buffer 0.02% sodium bicarbonate and 10% fetal calf serum.

Equal volumes of D2V at a multiplicity of infection of 0.05 and dilutions of a human dengue-1 immune serum (A. R.) were mixed and incubated for 30 min at room temperature. Homologous plaque reduction neutralizing ti-

ter of this serum was 1/40 and its D2V infection-enhancing titer, 1/1280. Next, mixtures were added to PBL cultures. Leukocyte suspensions were cultured in duplicates in 24-well plastic plates for three days at 37 °C in 5% CO₂. Virus produced in culture fluids was measured by quantitating dengue virus PFU in BHK21 cell monolayers (Morens et al., 1985).

ADE was determined by comparing virus in PBL culture with and without enhancing antibody and by determining a statistically significant difference in virus production using Detre's formula

$$\frac{X_1 - X_2}{\sqrt{X_1 + X_2}} \geq 1,96$$

where x₁ = total plaque numbers in cultures containing antibody and x₂ = total plaque counts in the same number of control cultures (Detre & White, 1970).

Five dengue 1 serum dilutions (1/640 to 1/5120) were tested for their ability to enhance infection of D2V in human PBL from BA patients and controls. Four enhancement parameters were measured: (a) significantly virus enhanced production at any serum dilution as determined by Detre's formula, (b) the highest dilution of serum producing significant infection enhancement (ENT); (c) the serum dilution producing greatest infection enhancement (peak enhancing titer, PENT); (d) power of enhancement obtained by dividing mean peak plaque counts in test cultures by the mean plaque counts in controls at the same dilution (Kliks et al., 1989).

RESULTS

In 27 out of 30 BA patients (90%) but only 7 of 13 controls (53.8%) significant D2V infection enhancement was observed in PBL (p < 0.05) by Fisher's Exact test (Table III).

TABLE III

Risk analysis of enhancing activity

D2V enhancement	Asthmatic group	Control
+	27	7
-	3	6

odds ratio = 7.71.

Mean D2V Ent and Pent titers were higher in the BA group compared with controls (χ^2 $p < 0.05$) (Table IV). Power of enhancement was higher in asthmatic patients.

TABLE IV

Comparison of D2V enhancing activities between asthmatic patient and control group

Group	Ent ^a	Pent ^a	Power ^a
Asthmatic (n = 30)	3.0 ± 1.0	2.7 ± 0.9	7.7 ± 9.0
Control (n = 13)	1.7 ± 1.6	1.6 ± 1.6	4.7 ± 7.2
p value	p < 0.01	p < 0.05	p > 0.05

a: log 10 value. Ent: enhancement titer; Pent: peak enhancement titer; Power: Power or fold of enhancement.

The odds ratio of PBL from asthmatic patients supporting D2V growth enhanced by antibody was 7.71 times higher than that of control PBL (χ^2 $p < 0.05$).

DISCUSSION

It has been suggested that chronic diseases may contribute to enhanced severity as possible risk factor of dengue illness in southeast Asia (Halstead, 1982). In the 1981 Cuban epidemic, BA was significantly associated with DHF/DSS patients: 25% of hospitalized children and 23% of fatal cases. These figures were twice as high as prevalence rates in the open Cuban population (1983 national survey) (Table V). Differences were statistically significant (χ^2 $p < 0.01$) (Bravo et al., 1987; Guzmán et al., 1987).

TABLE V

Bronchial asthma in three groups of patients with DHF/DSS and in the general Cuban population^a

Patients ^b	No. Pos/ total observations (%)	General population (%)
Children with DSS	19/76 (25)	11
Children fatal cases	16/71 (23)	11
Adults fatal cases	3/23 (13)	7

a: Bravo J (1987).

b: patients with previous history/total of patients (%).

Epidemiological evidences support the hypothesis that DHF/DSS occurs in persons with antibodies to one dengue serotype who are infected by a second type. Several *in vitro* and *in vivo* studies provide evidence that the replication of dengue virus in human peripheral blood leukocytes may be enhanced in the presence of subneutralizing concentrations of heterotypic dengue antibody. This phenomenon is known as antibody-dependent enhancement (Halstead et al., 1980; Halstead, 1982, 1988; Porterfield, 1985, 1986). If ADE plays an important role in the pathogenic mechanism of DHF/DSS we reasoned that D2V replication in PBL might differ from asthmatic patients compared with healthy persons.

Some authors have observed that virulence differences between dengue 2 strains are associated with subtle antigenic differences that affect the degree to which strains form immune-complexes with heterotypic dengue antisera (Morens et al., 1987; Morens & Halstead, 1987). To avoid confusing variables we used only one virus, a dengue 2 strain isolated during the 1981 DHF/DSS epidemic which probably contains common epitopes with the dengue 1 Cuban strain which circulated during the 1977 epidemic and a single enhancing antibody. These reagents are capable of producing ADE in P388D1 cells.

Halstead et al. (1980) reported considerable variability in replicate measurements of enhancing antibody. For this reason we used four dilutions of the dengue 1 immune serum for each PBL suspension studied, and used four parameters to measure ADE. In 18 cases the experiment was repeated twice and similar results obtained.

Halstead (1982) suggested that other mechanism besides infectious immune complexes might regulate virus infection of mononuclear phagocytes. These include, an increase in the size of the mononuclear phagocyte population permissive to dengue infection, an increase in the rate of immune phagocytosis or an increase in the yield of infectious virions per infected cells.

Here we report a statistically significant difference in virus replication in PBL from asthmatic patients compared with healthy individuals.

The question to be answered now, is: Why should ADE be greater in asthmatic patients?

A possible answer is the report that macrophage from asthmatic patients had significantly more Fc receptors for IgE and possibly for IgG than did controls (Joseph et al., 1983). Gin & Kay (1985) observed that patients using inhalents (bronchodilators alone or in combination with inhaled corticosteroids) circulating monocytes with higher FcR and complement rosettes than normals. In Cuba, inhalant therapy is very common in BA.

If macrophages from patients with BA have higher numbers of Fc receptors we can assume that the macrophages from these patients are more permissives for the penetration of the dengue virus and consequently the infection could be more efficient and resulting a higher number of infected cells.

Higher permissiveness of asthmatic patients macrophages supports the role of BA as a risk factor for DHF/DSS. This relation mechanism is still unknown although a genetic linkage, and stimulation of macrophages by drugs, or by disease produced factors, can be evoked.

There are some epidemiological data that suggest an underlying genetic mechanism in the development of the clinical picture of DHF/DSS.

During the 1981 Cuban epidemic white race was observed as a risk factor for DHF, even more, chronic diseases such as diabetes mellitus and sickle cell anemia (besides BA) were also risk factors for the severe disease (Bravo et al., 1987; Morier et al., 1987).

By these observations, it's not possible to exclude a genetic linkage that explains the role of BA in DHF/DSS, however influence of therapy or other humoral factors must be considered carefully.

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