

Breakdown of the blood–brain barrier during dengue virus infection of mice

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A breakdown of the blood–brain barrier occurred in mice inoculated intracerebrally (i.c.) or intraperitoneally (i.p.) with dengue virus type 2 (DEN2). This resulted in leakage of protein-bound Evans blue dye and ⁵¹Cr-labelled erythrocytes into the brain tissue. The leakage increased with time after infection and coincided with an increase of a DEN2-induced cytokine, the cytotoxic factor (CF), in the spleens of such mice. The titres of virus in the brain increased exponentially in i.c. inoculated mice but the virus was not detected in brains of mice given DEN2 by the i.p. route. Similar breakdown of the blood–brain barrier also occurred in mice inoculated intravenously with CF; the damage was dose-dependent and the vascular integrity was restored during the 3 h period after

inoculation. Treatment of mice with antihistamine drugs, blocking H1 or H2 receptors, decreased the DEN2-induced protein leakage by up to 50% in i.c. inoculated mice and up to 92% in those inoculated i.p. Indomethacin, a prostaglandin synthetase inhibitor, had no effect. In i.c. inoculated mice protein leakage was inhibited by about 60% by treatment with CF-specific (CFA) or DEN2-specific antisera (DEN2A) whereas protection was complete with the combined treatment with both antisera. On the other hand, in i.p. inoculated mice the inhibition of protein leakage was 80 to 89% with CFA. These findings show a breakdown of the blood–brain barrier leading to cerebral oedema during DEN2 infection which is mediated via the release of histamine by a virus-induced cytokine.

Introduction

Dengue viruses (DEN) generally cause a benign syndrome, dengue fever (DF), which is characterized by the acute onset of severe headache, myalgia and fever lasting for 3 to 7 days and a maculopapular rash appearing towards the end of the illness. The virus also produces a severe syndrome, dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS), which is characterized by increased vascular permeability and abnormal haemostasis (Halstead, 1980, 1988). Extensive biochemical changes occur in the enzymes and metabolites of skeletal muscles during DEN infection of mice which may explain the origin of myalgia (Agrawal *et al.*, 1978). Halstead (1980) put forward a hypothesis that a vascular permeability factor is produced in cases of DHF/DSS. Indeed, we know now that the vascular permeability is increased by two cytokines produced during DEN infection of mice: a T lymphocyte product, the cytotoxic factor (CF) and a macrophage cytotoxin (CF₂). Both CF and CF₂ kill macrophages and T helper cells by a Ca²⁺-dependent mechanism and increase vascular permeability by the release of histamine (Chaturvedi *et al.*, 1980; Khanna *et al.*, 1988, 1990, 1991; Dhawan *et al.*, 1990a, b, 1991).

During epidemics of dengue, cases of encephalitis have been observed. Two such cases have been reported

from this country (Sarkar *et al.*, 1969; Myers *et al.*, 1969) and others from Burma and Indonesia (reviewed by Sumarmo *et al.*, 1981). But from none of these cases could DEN be isolated from the brain tissue or cerebrospinal fluid (CSF); the virus was isolated only from the peripheral blood. DEN has been shown to lack the ability to invade the central nervous system (CNS) from the periphery (reviewed by Nathanson & Cole, 1970). Despite this, DF/DHF/DSS is characterized by severe frontal or retro-orbital headache, hallucination and depression, which indicates CNS involvement (Schlesinger, 1981). There are reports of definite development of cerebral oedema in such cases (S. B. Halstead in review by Sumarmo *et al.*, 1981). The cerebral oedema can be caused by vasogenic, cytotoxic or hydrocephalic mechanisms. In the present study, an effort was made to study the integrity of the blood–brain barrier during DEN infection of mice. It was observed that the blood–brain barrier suffered reversible damage caused by a virus-induced cytokine.

Methods

Mice. The study was carried out on inbred Swiss albino mice aged 3 to 4 months, obtained from the mouse colony maintained in this Department.

Virus. DEN type 2 (DEN2), strain P23085, was used in the form of an infected adult mouse brain suspension. The titre of the virus was estimated in the brain tissue of mice at different periods after inoculation of DEN2 intracerebrally (i.c.). A 10-fold serial dilution of each specimen was inoculated i.c. into groups of adult mice (at least six), and the titre was calculated by the method of Reed & Muench (1938). The titres have been expressed as \log_{10} LD₅₀ per 30 μ l of the brain suspension. The virus was inoculated in doses of 1000 LD₅₀. Normal mouse brain suspension (NMB) was prepared similarly for control groups.

Chemicals. The chemicals and reagents used in the study were Evans blue dye (Loba-Chemic Indo-austranal Co.), [⁵¹Cr]chromium as sodium chromate (Bhabha Atomic Research Centre), and indomethacin (Sigma).

Preparation and assay of cytotoxic factor (CF). CF was prepared from the spleen cells of DEN2-infected moribund mice (Charurvedi *et al.*, 1980) and was purified with a Pharmacia low pressure liquid chromatography system using a Sephacryl S-200 gel column. The amount of protein was estimated by the technique of Lowry *et al.* (1951). Normal mouse spleen homogenate (NH) was similarly prepared and used as a control. CF was concentrated by freeze-drying or by use of the Speed Vac (Savant Instrument). The cytotoxic activity of CF was assayed using a single-cell suspension of normal mouse spleen cells as the target. Doubling dilutions of CF were mixed in equal volumes with 2×10^6 target cells and incubated at 4 °C for 1 h (Chaturvedi *et al.*, 1980; Khanna *et al.*, 1989). The percentage of non-viable cells was calculated using the trypan blue dye exclusion method.

Preparation of antisera against CF. CF-specific antiserum (CFA) was prepared in rabbits as described elsewhere (Khanna *et al.*, 1990). CFA specifically neutralizes the cytotoxic activity of CF and reacts with it in a Western blot (Khanna *et al.*, 1990; M. Khanna & U. C. Chaturvedi, unpublished results). Sera obtained from normal rabbits were used as a control.

Preparation of antisera against DEN2. DEN2-specific antisera (DEN2A) were prepared by hyperimmunization of mice. A freshly prepared brain suspension (20%) from DEN2-infected mice was injected i.p. (0.2 ml) at weekly intervals for 5 weeks. DEN2A thus prepared was assayed for its capacity to neutralize the virus. Sera collected from normal mice were used as a control.

Assay of blood-brain barrier permeability. Permeability of the blood-brain barrier results in leakage of fluids, protein and cells into the brain substance. In the present study leakage of protein was assayed by measuring release of plasma protein-bound Evans blue dye into the brain whereas the leakage of cells was assayed using radiolabelled erythrocytes.

Evans blue dye method. Mice were inoculated intravenously (i.v.) with 200 μ l of a 2% solution of Evans blue dye dissolved in normal saline (0.85% sodium chloride). After 1 h the mice were anaesthetized with halothene i.p. and the brain was perfused with 5 ml normal saline through the left cardiac ventricle (Thumwood *et al.*, 1988). A 10% homogenate of the brain was prepared in normal saline and centrifuged at 4 °C at 6000 g for 15 min. The clear supernatant was collected and its absorbance was determined at 590 nm for the detection of Evans blue. The protein content of the fluid was calculated from a standard curve obtained with various concentrations of Evans blue coupled to bovine serum albumin estimated by the method of Lowry *et al.* (1951), and expressed as μ g protein/ml (Dhawan *et al.*, 1990a; Khanna *et al.*, 1990). The results were expressed as the permeability index (PI) of the blood-brain barrier which was calculated by comparison with the values obtained in control mice inoculated with NMB, after deducting background value, as follows: $PI = [100 \times (\text{protein in mice given DEN2} - \text{background value}) / (\text{protein in NMB-inoculated control mice} - \text{background value})] - 100$.

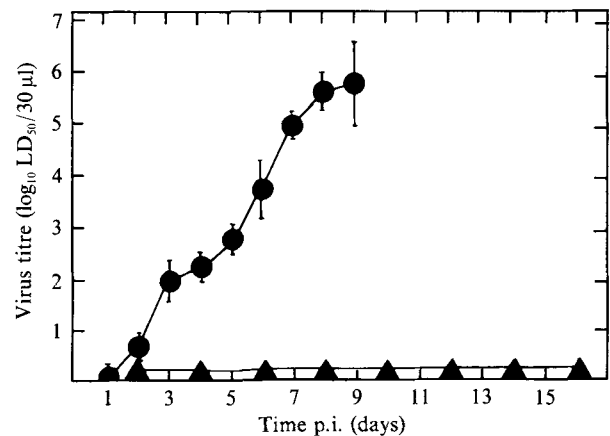


Fig. 1. Virus titres in the brain tissue of DEN2-infected mice on different days after i.c. (●) or i.p. (▲) inoculation. Each point represents mean \pm S.D. from three mice.

Radioisotope methods. In the second method, movement of radio-labelled mouse erythrocytes (MRBC) across the blood-brain barrier into the brain substance was monitored. Freshly collected MRBC were washed three times with phosphate-buffered saline (PBS) pH 7.2. The cells were counted and 3×10^9 MRBC/ml were incubated with ⁵¹Cr (1 mCi/ml as sodium chromate) for 30 min at 37 °C, with intermittent shaking. The cells were washed thoroughly with PBS, the cell count was adjusted and 100 μ l of the cell suspension was injected into the tail vein of mice. One hour later, mice were anaesthetized with halothene and the brains were removed after perfusion as described above. The ⁵¹Cr content of the whole brain was determined and the values were expressed as c.p.m./brain.

All the experiments were repeated and the mean \pm S.D. of the data obtained from eight to 12 mice are presented.

Results

Virus titre in the brain of mice

Following i.c. inoculation with DEN2, the mice appeared healthy up to day 5. On day 6 post-infection (p.i.), ruffling of the fur and arching of the back occurred which was followed by severe sickness with paralysis of limbs on days 8 and 9. All the mice died by day 11. Mice appeared to be unaffected after intraperitoneal (i.p.) inoculation of DEN2. Three mice were killed daily from days 1 to 9 after i.c. inoculation and on alternate days from days 2 to 16 after i.p. inoculation of DEN2 and their brains and spleens were collected. The brains were assayed for virus and the spleens for CF. The findings presented in Fig. 1 show that the virus titres in the brain increased exponentially; the titre was $5.8 \pm 0.8 \log_{10}$ LD₅₀ per 30 μ l of the brain suspension on day 9 in i.c. inoculated mice. In contrast no virus could be detected in the brain tissue in i.p. inoculated mice.

Production of CF in the spleen

The data presented in Fig. 2 show that in mice inoculated i.c. the cytotoxic activity appeared on day 5 and reached a peak on day 9 when 52 ($\pm 4\%$) of target cells were killed. In mice inoculated i.p. the peak of activity was seen on day 10 p.i.

Effect of DEN2 infection on blood-brain barrier

Fig. 3(a) shows a dark (deep blue) coloration of the brain tissue following i.c. inoculation of DEN2 due to leakage

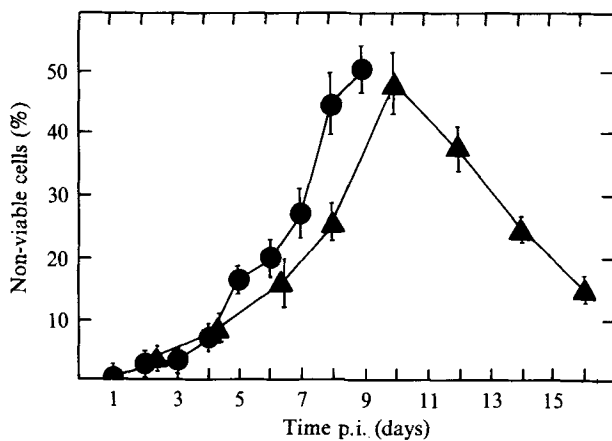


Fig. 2. CF titres in the spleen of DEN2-infected mice on different days after i.c. (●) or i.p. (▲) inoculation. Each point represents mean \pm s.d. from three mice.

of protein-bound Evans blue dye from the capillaries into extravascular spaces. In contrast, the brains of uninoculated mice or the control mice inoculated with NMB excluded the dye and did not show any blue colour. A bluish tinge was discernible from day 4 p.i. which gradually increased in intensity to become uniformly deep blue by day 9 p.i. in i.c. inoculated mice. None of the brains showed haemorrhagic spots. On the other hand, the brains of i.p. inoculated mice had only a bluish tinge from days 4 to 14 p.i.

The PI, assayed by protein dye leakage into the brains on different days after DEN2 inoculation is presented in Fig. 4(a). A sharp increase in PI occurred from day 7 and on day 9 it was 105 ± 10 in i.c. inoculated mice. On the other hand, in i.p. inoculated mice the protein dye leakage was maximal on day 10, when the PI was 59 ± 8 . At later periods it declined, being 15 ± 3 on day 16 p.i. (Fig. 4a). A similar pattern was observed when the leakage of radiolabelled MRBC into the brain tissue was assayed (Fig. 4b).

Effect of CF on blood-brain barrier

Mice inoculated with 250 μ g of CF i.v. were assayed for the integrity of the blood-brain barrier at different periods using the protein leakage method. The leakage of protein in the brain was maximal at 1 h; thereafter it declined and the integrity of the blood-brain barrier was restored after 3 h (Fig. 5). Therefore, in further experiments observations were recorded at 1 h after CF inoculation.

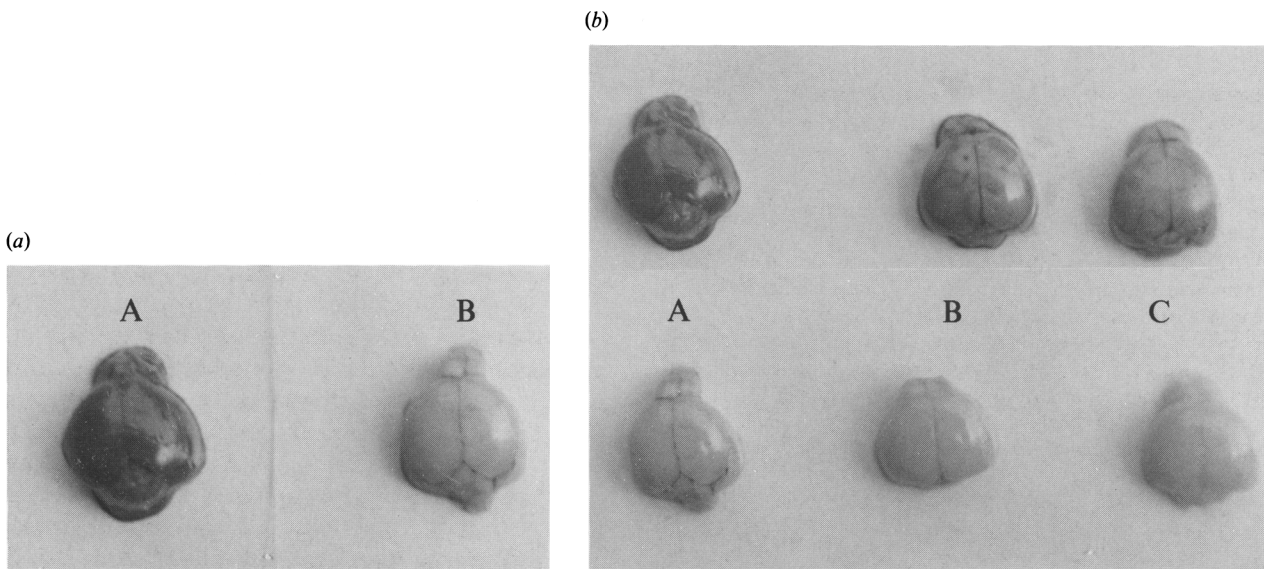


Fig. 3. Dark coloured appearance of brain following leakage of protein-bound Evans blue dye into the brain tissue. (a) Brains from DEN2-infected (A) and control (B) mice. (b) Brain from mice inoculated with 250 μ g (A), 200 μ g (B) or 100 μ g (C) of CF; brains in the lower row are from the control group.

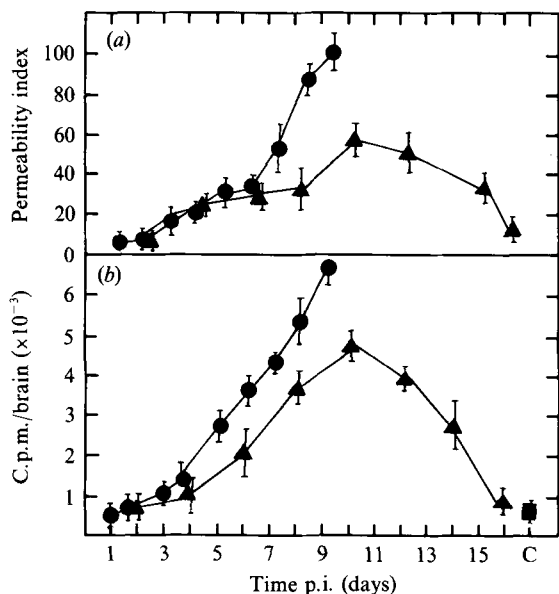


Fig. 4. Breakdown of blood-brain barrier in DEN2-infected mice. (a) Leakage of protein-bound Evans blue dye in brain tissue expressed as PI. (b) Leakage of ^{51}Cr -labelled MRBC in brain tissue expressed as c.p.m./brain. Symbols (●), i.c. inoculated; (▲), i.p. inoculated; (■), control mice inoculated with NMB. Each point represents mean \pm S.D. from 10 mice.

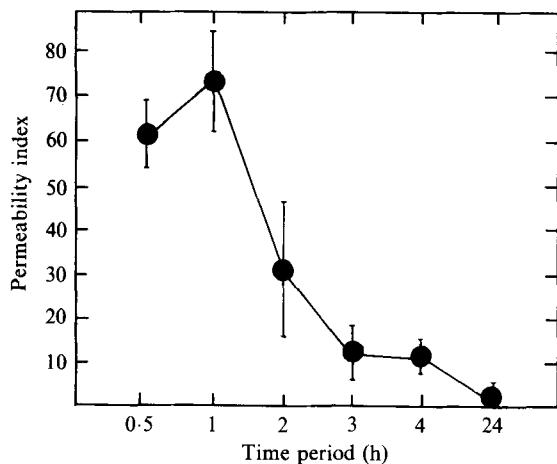


Fig. 5. PI of the brain tissue at different periods following i.v. inoculation of CF as determined by leakage of protein-bound dye. Each point represents mean \pm S.D. from 12 mice.

Groups of mice were inoculated with different doses of CF i.v. and for the controls, NH was inoculated i.v. After 1 h the integrity of the blood-brain barrier was assayed using Evans blue dye or radiolabelled MRBC. The effect of CF was dose-dependent as shown by the gross appearance of the brains (Fig. 3b) and after estimating the leakage of protein or radiolabelled MRBC in the brain tissue (Fig. 6).

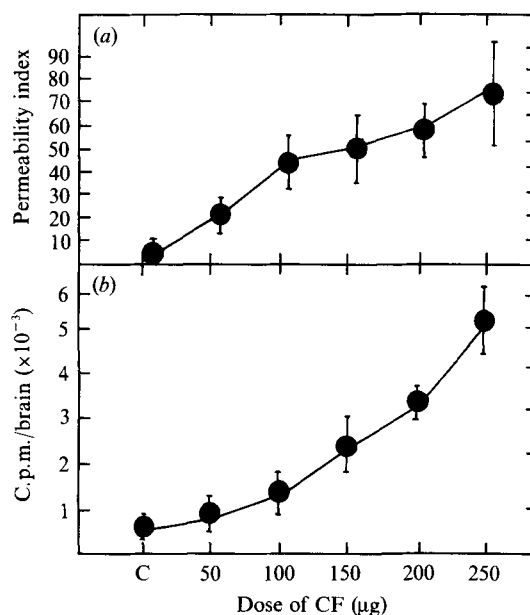


Fig. 6. Breakdown of blood-brain barrier in mice inoculated with different doses of CF. (a) Leakage of protein-bound Evans blue dye in brain tissue expressed as the PI. (b) Leakage of ^{51}Cr -labelled MRBC in brain tissue expressed as c.p.m./brain. Each point represents mean \pm S.D. from 10 mice.

Effect of antihistamine drugs

DEN2-infected mice were treated with 250 μg of avil (H-1 receptor blocker) or ranitidine (H-2 receptor blocker) i.v. on different days p.i. and the integrity of the blood-brain barrier was assayed using Evans blue dye after 1 h. The findings presented in Table 1 show that in i.c. inoculated mice the decrease in PI after avil treatment was 26 to 43% at different days and that with ranitidine was 16 to 37%. When DEN2 was inoculated i.p. the decrease in PI following treatment with the two antihistamine drugs was 57 to 92%.

In another set of experiments mice infected i.c. with DEN2 were treated i.v. with 250 μg of either of the drugs on days 4 and 8 p.i. and the integrity of the blood-brain barrier was assayed on day 9. It was observed that the PI was decreased by 49% by treatment with avil and 43% with ranitidine. The drug treatment had no effect on the clinical progression of the disease.

Effect of treatment with indomethacin

Cerebral oedema and breakdown of the blood-brain barrier has been reported to be mediated by prostaglandins in certain situations (Mohanty *et al.*, 1980). Mice were given 0.3 mg indomethacin i.p. 24 and 2 h before DEN2 i.c. or i.p. and then 0.1 mg i.p. at 48 h intervals (Shukla & Chaturvedi, 1981). The integrity of the blood-

Table 1. Effect of treatment with antihistamine drugs on the blood-brain barrier in DEN2-infected mice*

Time p.i. (days)	Groups	Avil		Ranitidine	
		Protein (µg/ml)	Decrease in PI (%)	Protein (µg/ml)	Decrease in PI (%)
I. DEN2 by i.c. route					
5	DEN2 + drug	665 ± 76	38	908 ± 89	16
	DEN2	1074 ± 117	0	1074 ± 117	0
7	DEN2 + drug	733 ± 68	26	754 ± 70	24
	DEN2	990 ± 77	0	990 ± 77	0
9	DEN2 + drug	952 ± 89	43	1052 ± 35	37
	DEN2	1677 ± 302	0	1677 ± 302	0
II. DEN2 by i.p. route					
10	DEN2 + drug	513 ± 60	57	370 ± 48	69
	DEN2	1193 ± 55	0	1193 ± 55	0
12	DEN2 + drug	357 ± 74	71	98 ± 48	92
	DEN2	1232 ± 109	0	1232 ± 109	0

* Mice inoculated with DEN2 were treated with 250 µg of avil or ranitidine i.v. on different days p.i. After 1 h, integrity of the blood-brain barrier was assayed using Evans blue dye. For background values mice were given the drug only. Each i.c. group consisted of 10 mice and the i.p. group of eight. The data have been presented after deducting background values. The decrease in the PI was calculated as follows: Decrease in PI (%) = [100 - (Protein in mice given DEN2 and the drug - background value) / (protein in mice given DEN2 - background value)] × 100.

Table 2. Effect of treatment of mice with indomethacin on the blood-brain barrier of DEN2-infected mice*

Time p.i. (days)	Protein (µg/ml)		Decrease in PI (%)
	Control	Drug-treated	
I. DEN2 by i.c. route			
7	1213 ± 142	1129 ± 113	7
9	1725 ± 310	1716 ± 281	1
II. DEN2 by i.p. route			
10	1132 ± 153	1075 ± 96	5
12	1280 ± 110	1242 ± 132	3

* Mice were treated with 0.3 mg indomethacin i.p. at 24 and 2 h before DEN2 inoculation i.c. or i.p. and then 0.1 mg doses of the drug at 48 h intervals. Each i.c. group consisted of 12 and i.p. group of eight mice. The integrity of the blood-brain barrier was assayed on the different days p.i. See Table 1 footnote for other details.

brain barrier was assayed on the different days p.i. The data presented in Table 2 show that treatment of mice with indomethacin had a negligible effect on the DEN2-induced permeability of the blood-brain barrier.

Effect of treatment with antisera

The effect of CF-specific antisera (CFA) on the blood-brain barrier of mice given DEN2 or CF was investigated. Groups of mice inoculated i.v. with 0.2 ml of various 10-fold dilutions of CFA were treated 24 h later with 200 µg of CF i.v. and the integrity of the blood-brain barrier

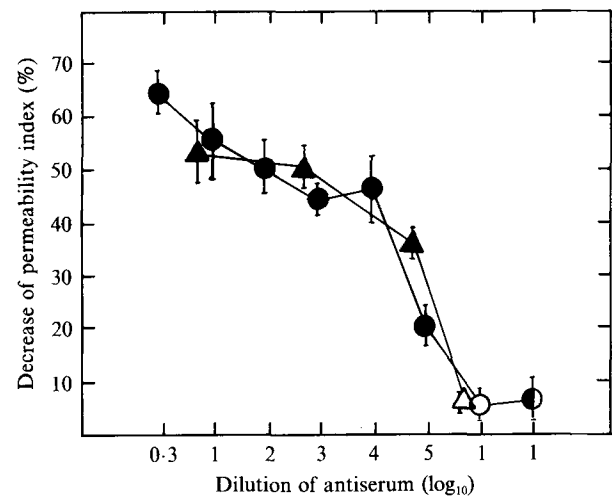


Fig. 7. Decreased PI by pretreatment of mice with specific antisera. Mice given CF i.v. were pretreated with CFA (●), or normal rabbit sera (○) or DVA (▲). Mice inoculated with DEN2 i.c. were pretreated with DVA (▲) or normal mouse sera (△). Each point represents mean ± s.d. from 10 mice.

was assayed after 1 h. Similarly, another group of mice given CFA were treated 24 h later with DEN2 i.c. and the blood-brain barrier was assayed on day 9 p.i. The findings presented in Fig. 7 show a dose-dependent decrease in the DEN2- or CF-induced leakage of protein in the brain. DEN2A had no effect on the CF-induced protein leakage.

Table 3. Effect of treatment with antisera on the DEN2-induced permeability of the blood-brain barrier*

Time p.i. (day)	Decrease in PI (%)					
	Timing of antiserum treatment					
	24 h before		Day 3 p.i.		Day 8 p.i.	
	CFA	DEN2A	CFA	DEN2A	CFA	DEN2A
I. DEN2 by i.c. route						
5	5 ± 3	15 ± 4	12 ± 2	17 ± 5	ND†	ND
7	3 ± 2	10 ± 3	28 ± 5	21 ± 3	ND	ND
9	52 ± 6	41 ± 7	48 ± 4	36 ± 6	60 ± 8	65 ± 5
II. DEN2 by i.p. route						
10	80 ± 4	32 ± 7	81 ± 9	28 ± 8	84 ± 9	22 ± 8
12	89 ± 8	28 ± 6	85 ± 12	30 ± 7	87 ± 10	26 ± 4

* Mice were treated i.v. with a 10^{-1} dilution of either CFA or DEN2A at 24 h before inoculation with DEN2 (given i.c. or i.p. route), or at days 3 or 8 p.i. For controls, mice were similarly given normal rabbit sera (for the CFA group) or normal mice sera (for the DEN2A group) and DEN2. For blank values, mice were given only the antisera. Each i.c. group consisted of 10 mice and each i.p. group of 8. The integrity of the blood-brain barrier was assayed on different days p.i. The decrease in the PI was calculated as shown in the Table 1 footnote.

† ND, Not determined.

Table 4. Effect of combined treatment with CF-specific and DEN2-specific antisera on the blood-brain barrier in DEN2-infected mice*

Groups	Timing of antisera treatment p.i. (day)	Protein ($\mu\text{g/ml}$)	Decrease in PI (%)
1	3	68 ± 20	96
2	3 and 8	117 ± 47	93
Control	Nil	1578 ± 237	0

* Mice of Group 1 inoculated i.c. with DEN2 were treated with CF-specific and DEN2-specific antisera (0.2 ml each of 10^{-1} dilution) i.v. on day 3, whereas those of Group 2 were given two doses of the above antisera, the first on day 3 and the second on day 8. Each group consisted of 10 mice. The integrity of the blood-brain barrier was assayed on day 9 after the virus infection. For other details and controls please see footnote to Tables 1 and 3.

The effect of the timing of the administration of CFA in relation to inoculation with DEN2 was investigated. Mice inoculated i.c. or i.p. with DEN2 received a 10^{-1} dilution of either of the antisera i.v. at 24 h before infection, or at day 3 or 8 p.i. The integrity of the blood-brain barrier was assayed on the different days p.i. and the data are presented in Table 3. It was observed that treatment with either of the antisera was most effective (the decrease in PI being up to 65%) when given on day 8 of the i.c. virus infection. Treatment with CFA had no effect on the outcome of infection, whereas mice treated with DEN2 up to day 3 p.i. did not develop any apparent illness or paralysis. In contrast, CFA provided a

significantly higher level of protection to the blood-brain barrier in i.p. inoculated mice, the decrease in the PI being 80 to 89% (Table 3).

In the mice inoculated i.c. with DEN2, neither CFA nor DEN2A could provide full protection against permeability of the blood-brain barrier; therefore, mice were treated with both the antisera. To one set of mice given DEN2 i.c., a 10^{-1} dilution of both CFA and DVA were given i.v. (0.2 ml of each), on day 3 p.i. To the second set of mice, two doses of both the antisera were given, one on day 3 and the second on day 8 p.i. The controls mentioned in previous experiments were included with each set. The integrity of the blood-brain barrier was assayed on day 9 p.i. The data summarized in Table 4 show that inhibition of PI was 96% when a single dose of the two antisera were given and 93% when two doses were given.

Discussion

The findings of the present study demonstrate that during DEN2 infection of mice, the blood-brain barrier is damaged resulting in leakage of protein-bound Evans blue dye and radiolabelled MRBC into the brain substance without any evidence of frank haemorrhaging. Similar damage to the blood-brain barrier was also produced, in a dose-dependent manner, by the inoculation of CF. The severity of the damage increased with time p.i., corresponding to the increased production of CF in the spleen. The exponential increase in virus titres

in the brain of i.c. inoculated mice and the failure to isolate DEN2 from the brains of i.p. inoculated mice is supported by previous findings (Chaturvedi *et al.*, 1978).

Brain capillaries are highly impermeable because their endothelium has tightly sealed junctions between individual cells that prevent the exchange of proteins across the vessel wall. Increased vascular permeability results in vasogenic oedema, principally in the white matter of the brain. Increased permeability of the blood-brain barrier could be due to damage to capillary endothelial cells or due to various vasoactive mediators which open up the junctions between cells. The findings of several experiments indicate that the damage to the blood-brain barrier in the present model is not morphological. These experiments are for example: the vascular integrity in CF-inoculated mice was restored by 3 h, administration of antihistamine drugs to DEN2-infected mice inhibited leakage of protein within 1 h, and administration of CF-specific antisera protected it. This is supported by a number of studies which did not show any evidence of injury to vasculature or the presence of the virus antigen or virus particles in the endothelial cells during DEN infection (Sahaphong *et al.*, 1980; reviewed by Halstead, 1988). A reversible induction of capillary permeability has been reported by administration of DEN2-induced CF/CF₂ in mice (Khanna *et al.*, 1990; Dhawan *et al.*, 1990a) which supports the present study.

It has been observed that CF/CF₂-induced capillary permeability is inhibited by antihistamine drugs in a dose-dependent manner, the inhibition being complete with a dose of 50 to 250 µg of the drugs (Khanna *et al.*, 1990; Dhawan *et al.*, 1990a). However in the present study, even after two doses of the drugs, inhibition of DEN2-induced permeability was no more than 50% in i.c. inoculated mice, whereas it was up to 92% in i.p. inoculated mice. Treatment of mice with indomethacin, an irreversible inhibitor of the enzyme prostaglandin synthetase (Vane, 1976), has no effect. Furthermore CFA completely protected mice against CF-induced increase in capillary permeability, even when the antibody was diluted 10⁻⁴ (Khanna *et al.*, 1990). This antibody at the 10⁻¹ dilution produced only a 60% decrease in PI in i.c. inoculated mice, despite the use of different schedules. The protection by CFA in i.p. inoculated mice was up to 89%. Mice given DEN2 by the peripheral route failed to show any symptoms because the virus does not invade the brain tissue, but a bell-shaped curve of CF titres is shown in the present study (Chaturvedi *et al.*, 1978; Dalakoti *et al.*, 1983). Grau *et al.* (1987) have shown that antibody against tumour necrosis factor prevents the development of murine cerebral malaria. Therefore, in the latter the presence of oedema, haemorrhage and leakage of protein across the blood-brain barrier due to cerebrovascular endothelial injury

may not be due entirely to the parasite itself. This is similar to the findings of the present study where CF is partly responsible for damage to the blood-brain barrier in i.c. infected mice.

Humoral antibody has been reported to play a critical role in recovery from primary DEN infection of mice. Adoptive transfer of specific antibody protected 50 to 100% of mice against i.c. challenge with DEN (Chaturvedi *et al.*, 1977, 1978). In the present study DEN2A protected DEN2-infected mice against sickness and paralysis but the effect on the blood-brain barrier was partial (65% protection). Furthermore, this antisera had no effect on CF-induced permeability of the blood-brain barrier. In contrast, the protection was complete when i.c. infected mice were treated with both CFA and DEN2A. Furthermore, in i.p. inoculated mice (where DEN2 does not invade the brain tissue) similar higher levels of protection were obtained with CFA. This showed that in the present model, the breakdown of the blood-brain barrier leading to the cerebral oedema was vasogenic and mediated by the DEN2-induced cytokine. Replication of the virus in the brain is the additional mechanism in i.c. inoculated mice.

In human cases of dengue the presentation which suggests CNS involvement is severe headache, hallucinations, depression and grief. Rarely, encephalitic signs and symptoms are also seen but DEN could be isolated only from blood and not from the brain tissue or CSF (Sarkar *et al.*, 1969; Myers *et al.*, 1969; reviewed by Sumarmo *et al.*, 1981). Pathological studies have shown frequent cerebral oedema, rarely haemorrhage but no evidence of encephalitis in such cases. A varying degree of cerebral oedema, even in benign dengue, may explain the origin of severe headaches. It is, therefore, likely that a cytokine, similar to that described here, may be one of the mechanisms of cerebral oedema in human cases of dengue. Clearly, more studies are needed to investigate this aspect in man.

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