
Dengue and dengue haemorrhagic fever: Indian perspective

U C CHATURVEDI* and RACHNA NAGAR

Department of Microbiology, CSM Medical University, Lucknow 226 003, India

*Address for correspondence: 201-Annapurna Apartments, No. 1, Bishop Rocky Street, Faizabad Road, Lucknow 226 007, India

*Corresponding author (Email, ucc05@rediffmail.com)

The relationship of this country with dengue has been long and intense. The first recorded epidemic of clinically dengue-like illness occurred at Madras in 1780 and the dengue virus was isolated for the first time almost simultaneously in Japan and Calcutta in 1943–1944. After the first virologically proved epidemic of dengue fever along the East Coast of India in 1963–1964, it spread to all over the country. The first full-blown epidemic of the severe form of the illness, the dengue haemorrhagic fever/ dengue shock syndrome occurred in North India in 1996. *Aedes aegypti* is the vector for transmission of the disease. Vaccines or antiviral drugs are not available for dengue viruses; the only effective way to prevent epidemic dengue fever/dengue haemorrhagic fever (DF/DHF) is to control the mosquito vector, *Aedes aegypti* and prevent its bite. This country has few virus laboratories and some of them have done excellent work in the area of molecular epidemiology, immunopathology and vaccine development. Selected work done in this country on the problems of dengue is presented here.

[Chaturvedi U C and Nagar R 2008 Dengue and dengue haemorrhagic fever: Indian perspective; *J. Biosci.* 33 429–441]

1. Introduction

Dengue viruses (DV) belong to family Flaviviridae and have four serotypes (1 to 4). They are transmitted mainly by the *Aedes aegypti* mosquito and also by *Aedes albopictus*. Biologically, DV are highly adapted to the mosquito and are maintained by vertical transmission. DV produces from a subclinical infection to a mild self limiting disease, the dengue fever (DF) and a severe disease that may be fatal, the dengue haemorrhagic fever/ dengue shock syndrome (DHF/DSS). The mosquito vectors are present in tropical and subtropical regions of the earth that determines the prevalence of DV in a region. Prior to 1970, only 9 countries had experienced cases of DHF; since then the number has increased more than 4-fold and continues to rise (figure 1a). The WHO published a global map of the distribution of the dengue epidemic activity during the year 2006 that shows whole India in red colour (figure 1b). A comparison with

similar maps prepared in earlier years shows that the activity of both the vector and the virus has spread to newer areas, acquiring global public health importance. An estimated 2.5 billion people in more than 100 countries are at risk of acquiring dengue viral infection with more than 50 million new infections being projected annually, 500000 cases of DHF that must be hospitalized and 20000–25000 deaths, mainly in children (Halstead 2002, 2007; Chaturvedi and Shrivastava 2004). Dengue has been an urban disease but now has spread to rural areas of India as well (Mehendale *et al* 1991; Kumar *et al* 2001; Arunachalam *et al* 2004; Tewari *et al* 2004). The factors considered responsible for Global resurgence of DF/DHF are unprecedented population growth, unplanned and uncontrolled urbanization, increased Air travel, absence of an effective mosquito control programme and deterioration of Public Health infrastructure. The risk factors for infection with DV are the increased density of the mosquito vector, reinfestation with *Ae. aegypti* of a

Keywords. Co-infection; dengue haemorrhagic fever; dengue virus; immunopathology

Abbreviations used: cytotoxic factor (CF); dengue fever (DF); dengue fever/dengue haemorrhagic fever (DF/DHF); DSS, dengue shock syndrome; dengue viruses (DV); T cells (TS)

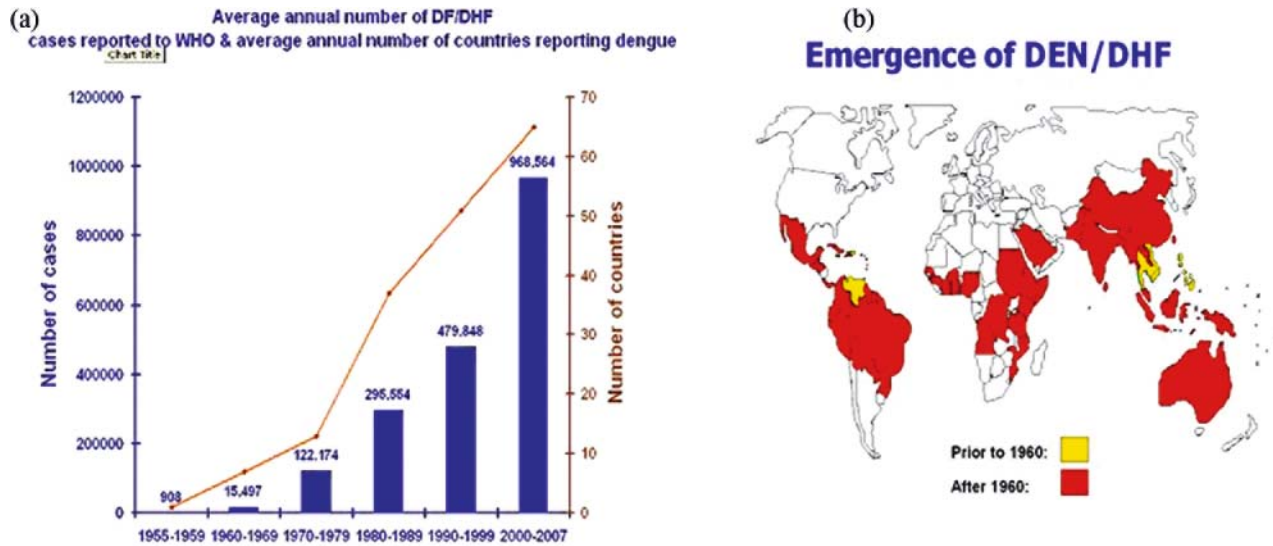


Figure 1. Global prevalence of dengue fever (DF) and dengue haemorrhagic fever (DHF) as shown by the WHO. **(a)** Average number of cases and the number of countries affected since 1955. **(b)** World map showing the prevalence of dengue virus before 1960 and during the year 2006. <http://www.who.int/csr/disease/dengue/impact/en/index.html>. Reproduced with permission of the WHO.

new geographical area, warm and humid climate, increased population density, water storage pattern in houses, storage of junk in open spaces, including tyres, coconut shells etc that trap rain water and introduction of new serotype of the virus, etc. Vaccines or antiviral drugs are not available for dengue viruses; the only effective way to prevent epidemic DF/DHF is to control the mosquito vector, *Ae. aegypti* and prevent its bite.

2. Dengue virus and immune response

DV is a positive-stranded encapsulated RNA virus and is composed of three structural protein genes, which encode the nucleocapsid or core (C) protein, a membrane-associated (M) protein, an enveloped (E) glycoprotein and seven non-structural (NS) proteins. Anti-E antibodies neutralize DV infectivity *in vitro* and protect mice from DV challenge on passive transfer and show a variable degree of cross-reactivity among the DV serotypes. Antibodies against NS1 can trigger complement-mediated lysis of DV-infected cells *in vitro* and protect mice from DV challenge. At the same time these antibodies may cross-react with endothelial cells leading to their activation and expression of cytokine, chemokine, and adhesion molecules resulting in cell damage. NS3 protein is the main antigen that stimulates DV-reactive CD4⁺ and CD8⁺ T cells that produce high levels of IFN- γ as well as TNF- α , TNF- β , and chemokines including macrophage inhibitory protein-1 β upon interaction with DV-infected antigen presenting cells, and are efficient at lysis of DV-infected cells *in vitro* (reviewed by Chaturvedi *et al* 2006a).

3. Clinical presentation

The patients initially develop an abrupt onset of high fever (39–40°C) with headache, retro-orbital pain, malaise, nausea, vomiting, and myalgia. The acute febrile stage lasts 2–7 days and may be followed by recovery but patients feel weakness. During defervescence some patients develop haemorrhagic manifestation that may be mild petechial haemorrhage, and bleeding at the nose, gastrointestinal tract and gums, which may be severe. Menorrhagia has been more prevalent due to the increasing number of affected adolescents, but haematuria is rare. Hepatomegaly is common with soft and tender liver. Thrombocytopenia and rising haematocrit due to plasma leakage are usually detectable before the onset of the subsequent stage of shock (DSS) with an abrupt fall to normal or subnormal levels of temperature, varying degrees of circulatory disturbances lasting for 24–48 h. In recent publications a number of atypical manifestations of dengue, such as encephalitis/encephalopathy (Kumar *et al* 2008) and myocarditis, hepatitis and cholecystitis etc. have been reported (Gulati and Maheshwari 2007). The majority of patients have rapid uneventful recovery without sequelae in the convalescent stage. The sequence of events in a patient after bite of infected mosquito is summarized in figure 2. The clinical diagnosis of DHF is based on four main characteristic manifestations (WHO 1997): (i) continuous high fever lasting 2–7 days; (ii) haemorrhagic tendency as shown by a positive tourniquet test, petechiae or epistaxis; (iii) thrombocytopenia (platelet count less than $100 \times 10^9/l$); and (iv) evidence of plasma leakage manifested by hemoconcentration (an increase in haematocrit 20%

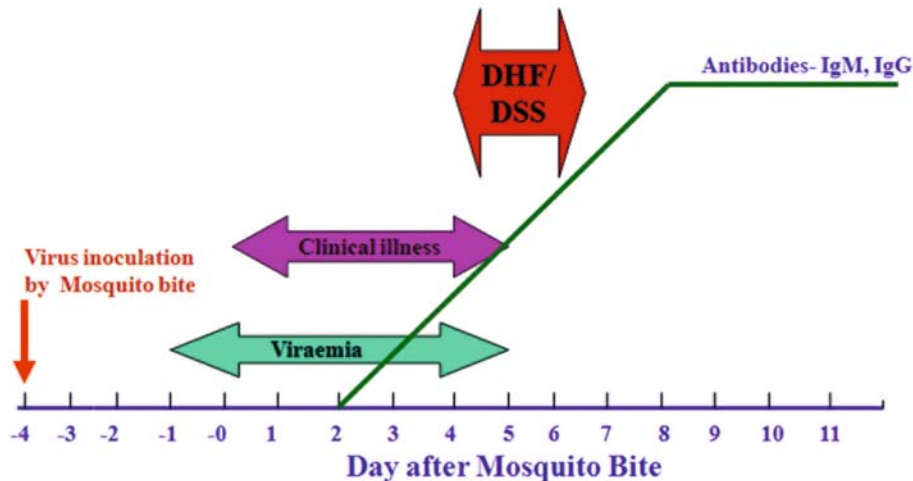


Figure 2. Sequence of events during dengue virus infection following the bite of infected mosquito.

above average for age, sex and population), pleural effusion and ascites etc. But some patients with bleeding or anemia may not have a rising haematocrit. Therefore, close observation, serial haematocrit and daily platelet count monitoring are suggested to fulfill the clinical diagnostic criteria. The severity of DHF is categorized into four grades (WHO 1997): grade I, being the mildest and grade IV being most severe, with circulatory failure manifested by a rapid and weak pulse with narrowing of pulse pressure (20 mmHg) or hypotension, with the presence of cold clammy skin and restlessness. There may be profound shock in which pulse and blood pressure are not detectable (DSS). In such patients the mortality rate is high.

4. Principles of laboratory diagnosis

Diagnosis of dengue virus infection is confirmed in the laboratory. During the stage of fever there is viraemia (figure 2) with presence of NS1 antigens in blood. The presence of virus in blood is detected either by isolation of the virus using infant mice or in tissue culture or by RT-PCR and the NS1 is detected by ELISA. During the post-febrile stage lasting a few weeks, IgM and IgG antibodies are present and are detected by Capture-ELISA (figure 2). During primary infection, viraemia and fever coincides, but during a secondary infection (second time infection with DV), the viraemia is present for 2 to 3 days, and NS1 antigens in blood lasts little longer. With a newer approach, artificial NS1 receptors have been implanted on a re-useable microchip that can capture and identify NS1 instantly and may be used for bedside diagnosis of dengue virus infection (Tai *et al* 2006).

5. Work done in India

This review is biased towards the work done in this country, reporting new virological/ pathological findings, efforts for the development of a vaccine or a diagnostic kit and are reflected in PubMed. Even then all the papers could not be cited due to constraint of space.

5.1 History of dengue

Dengue fever is an ancient disease. A clinical dengue-like illness was recorded in a Chinese medical encyclopedia in 992. With the expansion of shipping and growth of port cities in the 18th and 19th centuries the mosquito vector, *Ae. aegypti* and the dengue viruses spread to new geographic areas causing major epidemics. After the World War II, rapid urbanization in Southeast Asia led to increased transmission and hyperendemicity. The first epidemic of clinical dengue-like illness was recorded in Madras in 1780. Table 1 summarizes the occurrence of the epidemics of dengue-like illness in India. (Gubler 1997). Dengue virus was isolated in Japan in 1943 by inoculation of serum of patients in suckling mice (Kimura and Hotta 1944). The virus was isolated from sera of US soldiers at many parts of the World including Calcutta during 1944 (Sabin and Schlesinger 1945). The first virologically proved epidemic of DF in India occurred in Calcutta (now Kolkata) and Eastern Coast of India in 1963–1964 (Sarkar *et al* 1964; Chatterjee *et al* 1965; Carey *et al* 1966). The first major epidemic of the DHF occurred in 1953–1954 in Philippines followed by a quick global spread of epidemics of DF/DHF (Rigau-Perez *et al* 1998). DHF was occurring in the adjoining countries but it was absent in India for unknown reasons as all the risk factors were present. The DHF started simmering in

Table 1. Epidemics of dengue-like illness in India (before discovery of the virus)

Year	Places
1780	Madras
1824–1925	Rangoon to Madras
1844–1949	Kanpur, Calcutta
1852–1956	Wide spread
1870–1973	Bombay, Calcutta, Madras
1897–1999	Bombay
1901–2007	Madras
1907–1913	Calcutta, Pune, Meerut
1920–1926	Lucknow, Bombay, Calcutta
1927–1928	Coimbatore
1930–1933	Madras
1934–1936	Madras
1940–1945	Calcutta

various parts of India since 1988 (Kabra *et al* 1992; Chaturvedi U C, Mukerji R and Nagar R, unpublished data; Bhattacharjee *et al* 1993; Cherian *et al* 1994). The first major wide spread epidemics of DHF/DSS occurred in India in 1996 involving areas around Delhi (Dar *et al* 1999) and Lucknow (Agarwal *et al* 1999a) and then it spread to all over in the country (Singh *et al* 2000; Shah *et al* 2004).

5.2 Virological investigation of epidemics of DF/DHF

In this vast country virus laboratories are few and wide apart, therefore, large section of the population has no facilities for viral diagnosis. Now diagnosis by commercial kits is available to many hospitals. After the initial epidemic of dengue fever on the Eastern Coast of India in 1963–64 (Sarkar *et al* 1964; Chatterjee *et al* 1965; Carey *et al* 1966), it spread Northwards and reached Delhi in 1967 (Balaya *et al* 1969) and Kanpur in 1968 (Chaturvedi *et al* 1970a, b). Gradually the whole country was involved with wide spread epidemics followed by endemic/hyper-endemic prevalence of all the four serotypes of DV.

The epidemiology of dengue virus and its prevalent serotypes has been ever changing. The epidemic at Kanpur during 1968 was due to DV-4 (Chaturvedi *et al* 1970a) and during 1969 epidemic; both DV-2 and DV-4 were isolated (Chaturvedi *et al* 1972). It was completely replaced by DV-2 during 1970 epidemic in the adjoining city of Hardoi (Chaturvedi *et al* 1974). Myers *et al* (1968, 1969) had reported the presence of DV-3 in patients and *Aedes aegypti* at Vellore during the epidemic of 1966 while during the epidemic of 1968, all the four types of DV were isolated from patients and mosquitoes (Myers *et al* 1970). In another study Myers and Varkey (1971) reported an instance of a

third proved attack of DV in one individual. DV-2 was isolated during the epidemics of dengue in urban and rural areas of Gujrat state during 1988 and 1989 (Mahadev *et al* 1993). Outbreaks of dengue occurred in Rajasthan by DV-1 and DV-3 (Ghosh *et al* 1974), DV-3 (Chauhan *et al* 1990), Madhya Pradesh by DV-3 (Rodriguez *et al* 1973), Jammu by DV-2 (Padbidri *et al* 1996), Gujarat by DV-2 (Mahadev *et al* 1993) and in Haryana by DV-2 (Kumar *et al* 2001). DV-2 was the predominant serotype circulating in Northern India, including Delhi, Lucknow and Gwalior (Dar *et al* 1999; Agarwal *et al* 1999a; Parida *et al* 2002) while DV-1 was isolated during the 1997 epidemic at Delhi (Kurukumbi *et al* 2001). The phylogenetic analysis by the Molecular Evolutionary Genetics Analysis program suggests that the 1996 Delhi isolates of DV-2 were genotype IV. The 1967 isolate was similar to a 1957 isolate of DV-2, P9-122, from India, and was classified as genotype V. This study indicates that earlier DV-2 strains of genotype V have been replaced by genotype IV (Singh *et al* 1999). The Gwalior DV-2 viruses, were classified into genotype-IV, and were most closely related to Delhi 1996 DV-2 viruses and FJ 10/11 strains prevalent in the Fujian state of China. However, two earlier Indian isolates of DV-2 were classified into genotype-V. Genotype V of DV-2 has been replaced by genotype IV during the past decade, which continues to circulate silently in North India, and have the potential to reemerge and cause major epidemics of DF and DHF (Dash *et al* 2004).

DV-3 has been isolated during the epidemics at Vellore in 1966 (Myers *et al* 1968; 1969), at Calcutta in 1983 (Mukherjee *et al* 1987) and in 1990 (Bhattacharjee *et al* 1993), at Jalore city, Rajasthan in 1985 (Chouhan *et al* 1990) at Gwalior in 2003 and 2004 (Dash *et al* 2005, 2006). At Delhi, till 2003, the predominant serotype was DV-2 (genotype IV) but in 2003 for the first time all four dengue virus subtypes were found to co-circulate in Delhi thus changing it to a hyperendemic state (Dar *et al* 2003) followed by complete predominance of DV serotype 3 in 2005 (Gupta *et al* 2006). During the 2004 epidemic of DHF/DSS in Northern India a sudden shift and dominance of the DV serotype-3 (subtype III) occurred replacing the earlier circulating serotype-2 (subtype IV) (Dash *et al* 2006). Co-circulation of DV serotypes in Delhi in 2005 has also been reported by Saxena *et al* (2006), which may have implications for increased DHF/DSS. Kukreti *et al* (2008) have reported emergence of a distinct lineage of DV-1, having similarity with the Comoros/Singapore 1993 and Delhi 1982 strains, but quite different from the Delhi 2005 lineage and microevolution of the pre-circulating DV-3. Co-circulation of several serotypes of dengue viruses has resulted in concurrent infection in some patients with multiple serotypes of DV (Bharaj *et al* 2008). Concurrent infection by Chikungunya and DV-2 was reported by Myers and Carey (1967) from Vellore. In a recent study the economic burden faced by India during the

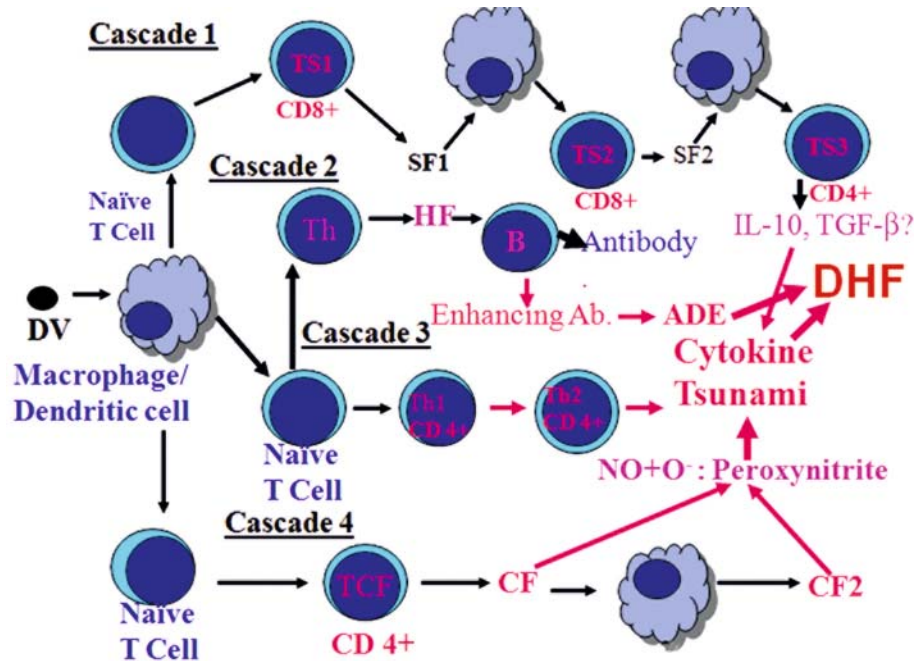


Figure 3. Immunopathogenesis of DHF via induction of four cascades of T cells as dissected out by U C Chaturvedi and colleagues. Please see the text.

2006 dengue epidemic was estimated and was found to be US\$27.4 million (95% CI US\$25.7–29.1 million) (Garg *et al* 2008).

5.3 Development of mosquito cell culture

In a landmark study, Singh and Paul (1969) reported development of a mosquito cell culture for the isolation of dengue viruses. This was the first time when mosquito cells were used as cell culture. This was a forerunner of the widely used mosquito cell line C6/36.

5.4 Pathogenesis of DHF

The pathognomonic features of DHF are increased vascular permeability without morphological damage to the capillary endothelium, thrombocytopenia, altered number and functions of leucocytes, altered haemostasis and liver damage. Extensive plasma leakage occurs suddenly in various serous cavities of the body including the pleura, pericardium and peritoneal cavities and associated haemorrhages, in patients with DHF grades III and IV may result in profound shock, the DSS. A lot of work done in the last several decades but the pathogenesis of DHF is not fully understood reviewed by (Chaturvedi *et al* 2006a, b, 2007). Among the several hypothesis proposed to explain the pathogenesis of severe

dengue disease, three are the landmark reports. First was the role of enhancing antibodies (Halstead 1970); the second was the demonstration of a shift from Th1-response in mild dengue to Th2-response in severe DHF (Chaturvedi *et al* 1999a) associated with the disturbed fine balance of the cytokine cascade resulting in a ‘Cytokine Storm’ that causes all the pathological lesions (Chaturvedi *et al* 1999a; 2000); and the third is presence of cross-reactive memory T cells that induce secretion of large amount of cytokines on a second exposure to DV (Mongkolsapaya *et al* 2003, 2006). DV-specific memory lymphocytes have been detected even 20 years after a primary infection with the virus (Sierra *et al* 2002). Whatever is the mechanism, it ultimately targets endothelium to produce pathological lesions and the severe dengue disease (Basu and Chaturvedi 2008).

A lot of work has been done experimentally and in patients in this country to understand the immunopathogenesis of DHF and the findings have been validated in the subsequent epidemics of DHF. Our findings summarized in figure 3 show that during DV infection four cascades of T cells are induced that might singly/jointly result in vascular leakage and DHF.

5.4.1 Suppressor T cell cascade: Cascade 1: For the first time a microbe-induced suppressor T cells cascade was delineated in DV-infected mice (Tandon *et al* 1979; Shukla and Chaturvedi 1981, 1983; Chaturvedi 1984) and now it

has been confirmed in a large number of viruses (Mills 2004). The conclusions drawn at that time using primitive techniques (Chaturvedi 1984) are valid even today using modern technique (Mills 2004). The antigen –specific suppressor T cell cascade in DV-infected mice consists of three generations of suppressor T cells (TS) and their secretory soluble suppressor cytokines (SF) with in between $M\phi$ transmitting the signals (figure 3, cascade I). DV-infected $M\phi$ transmit the signal to recruit TS1 cells, which secrete a suppressor cytokine, SF1. The suppressor signal of SF1 is transmitted via live syngeneic $M\phi$ to recruit a second subpopulation of suppressor T cells (TS2), which produce another soluble, prostaglandin-like suppressor cytokine (SF2). SF2 induces production of a third subpopulation of suppressor T cells (TS3), which suppresses humoral immune response in an antigen-specific and genetically restricted manner (Shukla and Chaturvedi 1981, 1982, 1983; Chaturvedi 1984). Of two polypeptide chains of SF1 (Bhargava *et al* 1990), α -chain bind to the α -chain of the SF receptor sites (SF-R) on present on $M\phi$ (Mukherjee *et al* 1993a) while the β -chain of SF binds to H-2A determinants on $M\phi$ (Mukherjee *et al* 1993b; 1994). Internalized SF is degraded, binds to H-2K antigen and is transported to a site other than SF-R on $M\phi$ membrane for recruitment of TS2 cells (Tripathi *et al* 1997). DV-induced suppressor pathway suppresses antigen-specific antibody production including that of enhancing antibody. Thus increased replication of the virus mediated by the enhancing antibody and the immunopathology mediated by the immune-complex is prevented. On the other hand, suppression of neutralizing antibody would delay elimination of DV from the body causing pathological lesions (Chaturvedi *et al* 1984, 2007). Suppressor T cell activity, regulating B and T cell response in dengue type 3 virus-infected mice has been shown (Nagarkatti and Nagarkatti 1983).

5.4.2 DV-specific antibody cascade: Cascade 2: As shown in figure 3 (cascade 2) DV induces generation of helper T cells in mouse spleen which enhance the antigen-specific antibody plaque forming cell count in syngeneic mice (Chaturvedi *et al* 1987). The DV-specific helper T cells secrete a soluble cytokine, the helper factor (HF) (Chaturvedi *et al* 1992a) which is a disulphide bonded double chain structure, one chain having antigen and the other having I-A determinants; the presence of both chains is essential for helper activity (Chaturvedi *et al* 1991b). HF binds to the surface of macrophage and the helper signal is transmitted only by a close physical contact of the plasma membranes of the signal presenting cells (helper T cell or HF-adsorbed macrophage) and B cells (Chaturvedi *et al* 1992b; Rizvi *et al* 1993). Th and HF helped increased activity of B cells results in production of DV-specific antibodies that are protective; and also production of enhancing antibody that mediate

antibody dependent enhancement (ADE) of infection resulting in DHF (figure 3, cascade 2).

5.4.3 Helper T cell cascade, cascade 3: Helper cell type-1 (Th1) cells secrete interferon-gamma (IFN- γ), interleukin-2 (IL-2) and tumour necrosis factor- α (TNF- α) and are responsible for cell-mediated inflammatory reactions, delayed type hypersensitivity, tissue injury in infections and autoimmune diseases. Th2 cells secrete IL-4, IL-5, IL-6, IL-10, and IL-13 and are associated with help for B cell antibody production. Cross-regulation of Th1 and Th2 is primarily mediated by IL-10 and IFN- γ respectively. In a number of parasitic, fungal, bacterial and viral infections such as human immunodeficiency virus, herpes simplex and influenza viruses, a Th1 response is linked to recovery from infection while a Th2-type response tends to lead to severe pathology and exacerbation of the disease (reviewed by Mosmann and Sad 1996). A balanced helper T cell-type 1 (Th1) cytokine response and Th2-type cytokine response controls immune response to microbe. During the epidemic of DHF at Lucknow during 1996 the pattern of above cytokines and their mRNA were studied. For the first time it was reported that a shift from predominant Th1 response seen in patients with DF to Th2 response (figure 3, cascade 3) in severe dengue disease (Chaturvedi *et al* 1999a). By suppressing protective Th1 responses the regulatory T cells may enhance the Th2 response and increase the infection-induced. Both, the regulatory T cell and Th2 cell secrete IL-10 and TGF- β (Agarwal *et al* 1999b) thus disturbing the fine balance in the different cytokines causing a “Cytokine Tsunami” and severe dengue disease figure 3, cascade 3).

5.4.4 Cytotoxic cascade, cascade 4: A unique cytokine, cytotoxic factor (CF) which is produced by CD4⁺ T cells during dengue virus infection of mice and man. The amino-terminal sequence of CF has no homology with any of the known proteins. Majority of the patients with dengue show the presence of CF in their sera, with peak amounts in the most severe cases of DHF grade IV (Chaturvedi *et al* 1999b). CF selectively kills CD4⁺ T cells and H-2A⁺ macrophages ($M\phi$) and induces H-2A⁺ $M\phi$ to produce another cytokine, the $M\phi$ cytotoxin (CF2) that amplifies the effect of CF (figure 3, cascade 4). CF was detected in about 70% of the stored sera from patient with dengue at Bangkok at AFRIMS (Mukerjee *et al* 1997) and later at Lucknow, India during the first extensive epidemic of DHF in India in 1996. CF was purified from the pooled sera of the DHF patients. On intravenous inoculation into mice it increased capillary permeability and damaged the blood-brain barrier, indicating its role of CF in the pathogenicity (Mukerjee *et al* 1997). CF and CF2 may be pathogenesis-related proteins, capable of reproducing DHF-like pathological lesions in mice (figure 3, cascade 4), such as increased

capillary permeability, cerebral edema, and blood leukocyte changes (Chaturvedi *et al* 1991a; 1997). CF is produced in *ex vivo* cultures of CD4⁺ T cells obtained from peripheral blood of the patients with severe dengue disease (Agarwal *et al* 1998). Human peripheral blood leucocyte cultures inoculated with DV produce CF (Chaturvedi *et al* 1999c). The production of CF precedes the clinical illness and is present in 100% patients with DF/DHF upto the 4th day of illness, then declines and is not detectable after the 20th day of illness (Chaturvedi *et al* 1999b). Since CF is DV-specific it can be used for developing a diagnostic kit. The DHF-like pathological lesions produced by CF/CF2 can be prevented by pretreatment of mice with the anti-CF antibodies. Further, active immunization of mice using CF as antigen protects them against subsequent challenge with CF. Challenge of such mice with a lethal intracerebral dose of DV results in death without appearance of clinical symptom of the disease (Chaturvedi *et al* 1994). CF/CF2 induce M ϕ to produce free radicals, nitrite, reactive oxygen and peroxynitrite (Misra *et al* 1996a, b; 1998; Chaturvedi *et al* 1997, 2000, 2006a). The free radicals, besides killing the target cells by apoptosis also directly upregulate production of proinflammatory cytokines IL-1 β , TNF- α , IL-8, and hydrogen peroxide in M ϕ (figure 4). Oxidative stress develops since early days of onset of dengue infection. Plasma protein carbonylation, protein carbonylation to protein-bound sulphhydryl group ratio are reported to predict DHF/DSS (Soundravally *et al* 2008). The change in relative levels of IL-12 and TGF- β shifts a Th1-dominant response to a Th2-biased response resulting in an exacerbation of dengue disease. The vascular permeability is

increased due combined effect cytokine tsunami, release of histamine, free radicals and the products of the complement pathway etc. Thus the key player appears to be CF/CF2, but their activity is regulated by CF-autoantibodies generated (figure 4) in patients with dengue disease (Chaturvedi *et al* 2001).

5.5 Effect of DV infection on megakaryocytes and platelets

One of the classical features of DF/DHF is thrombocytopenia. The occupational and non-occupational exposure to toxic hexavalent chromium Cr (VI) is common so is the infection with DV in this country. Therefore, the effects of DV infection on peripheral blood cells of mice fed Cr (VI) with drinking water (for 3 to 9 weeks) were investigated. It was observed that in Cr (VI) fed mice the DV-induced thrombocytopenia was abrogated (Shrivastava *et al* 2005). Trivalent chromium picolinate (CrP) is used worldwide as micronutrient and nutritional supplement. Another study was therefore, carried out to investigate the effects of CrP on various haematological parameters during DV infection of mice. The findings showed that the adverse effects of DV infection, especially on platelets and leucocytes, were abrogated by pretreatment of mice with CrP (Shrivastava *et al* 2007). The therapeutic utility of CrP in viral infections including dengue needs to be studied in depth. In a study, Basu *et al* (2008) examined the effect of DV-2 on the *in vitro* growth and differentiation of thrombopoietin-induced megakaryopoiesis of cord blood CD34⁺ cells.

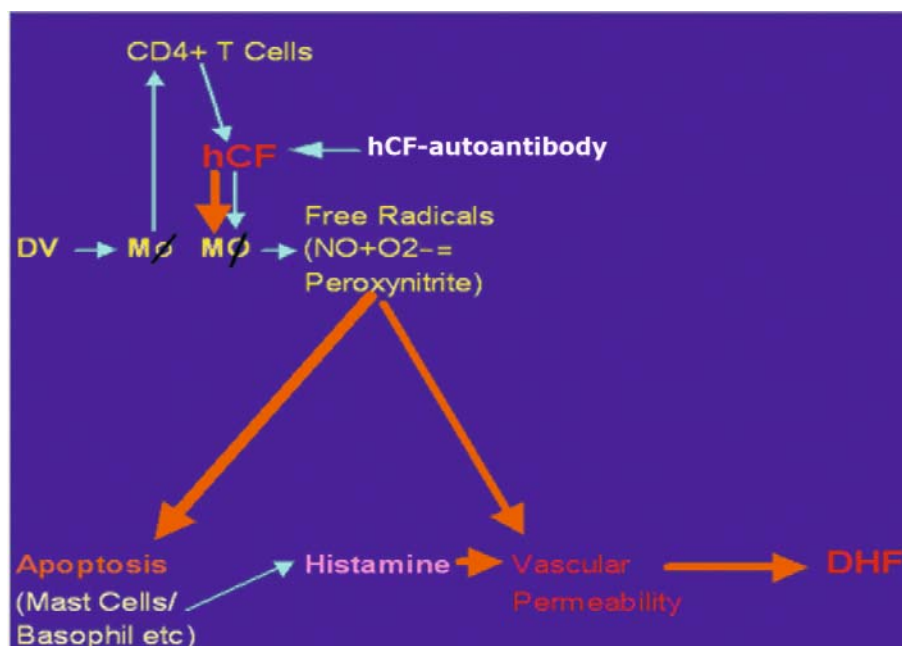


Figure 4. Increased vascular permeability mediated by the cytotoxic factor. Please see the text.

They found that DV-2 inhibits *in vitro* megakaryopoiesis and induce apoptotic cell death in a subpopulation of early megakaryocytic progenitors. These events might contribute towards the origin of thrombocytopenia in dengue disease. In another study they show that DV-2 exposure can activate platelets with increase in P-selectin expression and fibrinogen-binding property. Atomic force, scanning and transmission electron microscopy also showed typical activation-related morphological changes such as altered platelet membrane architecture, degranulation, and presence of filopodia and dilatation of the open canalicular system in the DV-2-exposed platelets but not in the controls. This suggests that DV-2 may directly interact with and activate platelets and thus may be responsible for thrombocytopenia (Ghosh *et al* 2008).

5.6 Influence of host genetics on dengue disease

The profound influence of the host's genetic makeup on susceptibility/resistance to infections has been established in numerous studies. In a review paper the existing knowledge dealing with the host genetics and its implication in the pathogenesis of the severe dengue disease is presented (Chaturvedi *et al* 2006b). The polymorphic transporter associated with antigen processing (TAP) 1 and TAP2 genes encode subunits of the transporter that delivers peptides to the human leukocyte antigen class I molecules. Soundravally and Hoti (2007a) have reported that TAP1 gene polymorphism is associated with severe dengue infection. In another report on TAP 2 gene polymorphism in dengue, they have suggested that heterozygous pattern at TAP2 379 locus confers susceptibility to DHF, and TAP2 665 THR/ALA genotype was found to be a risk factor for development of DHF (Soundravally and Hoti 2007b).

5.7 Development of diagnostic kits

Development of a quick, sensitive, specific and cheaper diagnostic test is an urgent need.

A number of kits are commercially available for the diagnosis of DV infection but they need strict quality control. The National Institute of Virology, Pune is producing a kit and is supplying all over India. Work is going on in some institutions of India to develop a better kit. Parida and colleagues have developed 'dip-stick' diagnostic ELISA kits for the diagnosis of DV infection in the field situations (Parida *et al* 2001; Abhyankar *et al* 2006). They have also developed fusogenic peptide as diagnostic marker for detection of flaviviruses (Pattnaik *et al* 2006). Anandarao *et al* (2005) developed a customized recombinant dengue multiepitope protein (r-DME-G) that can specifically detect the IgG class of antidengue antibodies in patient sera. Using

this strategy, they have created another dengue multiepitope protein, r-DME-M, with specificity for the IgM class of antidengue antibodies. The purified protein was used to develop an IgM ELISA and has been adapted to a rapid strip test format (Anandarao *et al* 2006). Further, r-DME-G and r-DME-M proteins have been produced in bulk (Tripathi *et al* 2007a, b). This approach may circumvent the drawbacks of the whole virus antigen-based commercial kits. Hapugoda *et al* (2007) have designed a novel tetravalent chimeric antigen fusing envelope domain III of each of the four DV types and have demonstrated that this tetravalent antigen can function as a diagnostic tool of high sensitivity and specificity. The serodiagnosis of DV infection is confirmed by RT-PCR and Nested PCR that have been further improved by real time RT-PCR and Multiplex-PCR. Parida *et al* (2005) have developed a gene based amplification technique, which they call, RT-LAMP. Recently, Saxena *et al* (2008) have developed a single step Multiplex-RT-PCR for the detection and typing of dengue viruses.

5.8 Development of dengue virus vaccines

DV was discovered more than sixty years back but we do not have an effective vaccine against it, which indicates problems in its development. The problems are several, e.g. the pathogenesis of DHF is not fully known, and absence of an animal model for the dengue disease and pre-existing heterotypic dengue antibody is a risk factor for DHF. Therefore, a tetravalent vaccine that prevents infection with all four DV serotypes is needed. Natural DV infection induces long-lasting protective immunity only to the same serotype. A tetravalent formulation that retains the immunogenicity of all four serotypes has proven difficult, requiring the use of more complicated, multiple-dose immunization regimens. A detailed discussion on this problem is presented elsewhere (Chaturvedi *et al* 2005). In spite of the problems, the surge in publications on the development of dengue vaccines based on new generations of biotechnology techniques, indicate the profound interest and urgency for combating the dengue disease. The efficacy and safety of some of the new vaccine candidates have been evaluated and proven in human preclinical/ clinical trials (Chaturvedi *et al* 2005). Only the Indian initiative in this field is presented here. In recent years, the carboxy-terminal region of the major dengue virion envelope (E) protein, known as domain III (ED III), has emerged as a significant sub-unit vaccine candidate. In a novel approach, Jaiswal *et al* (2004) have expressed and purified recombinant domain EDIII that refolds *in vitro* and protects cells in culture against DV-2 infection by blocking the virus from binding to host cells. Khanum *et al* (2006) have created a recombinant adenovirus capable of expressing the EDIII of DV-2 and tested it in combination with a plasmid encoding the same

domain to determine its potential as a possible dengue vaccine candidate. Using two regimens they have shown induction of neutralizing antibodies and Th1 type response. Further, they have described construction of a recombinant, replication-defective Ad (rAd) vector encoding a chimeric antigen made of in-frame linked EDIII of DV serotypes 2 and 4. Using this rAd vector, in conjunction with a plasmid vector encoding the same chimeric bivalent antigen, in a prime-boost strategy, they have shown that it is possible to elicit equipotent neutralizing and T cell responses specific to both DV serotypes 2 and 4 (Khanam *et al* 2007) This work has implications for the development of safe and effective tetravalent dengue vaccines.

6. Conclusions and future perspectives

Dengue virus infection is endemic in India with frequent epidemics of DF/DHF. All the four serotypes are circulating resulting in concurrent infection with two DV. In the last few years the predominant serotype is DV-3. The vector has adapted to extremes of warm and cold weather resulting in occurrence of dengue cases round the year. Dengue and the vector have been reported in the arid zones of Rajasthan (Chouhan *et al* 1990; Angel and Joshi 2008). There are large number of reports on atypical clinical presentations but they are not backed by virological studies due to lack of such facilities at most parts of the country. There is no adequate animal model for DHF and a number of hypotheses have been put forward to explain the vascular leakage. With massive efforts all over the world the vaccine is round the corner. The problem of dengue is mammoth in this country that is compounded by the huge population, poor medical and diagnostic facilities, inadequate mosquito control and all the ground conditions that favour expansion of the vector. This country needs a large number of virus laboratories that may provide quick and reliable diagnosis. Efforts will have to be made to develop improved, proactive, laboratory-based surveillance systems that can forecast impending dengue epidemics. This will alert the public to take action and physicians to diagnose and properly treat DF/DHF cases. There is need to have a strong epidemiology/pathology/experimental pathology backup. Are our efforts adequate? Our efforts are limited only to the stage of crisis management. We need dedicated teams to solve the problems and minimize the human suffering.

Acknowledgements

We thank Dr. Cecilia Dayaraj, Division of Dengue Virus Research, National Institute of Virology, Pune for critically reading the manuscript and for providing the work done at her Institute. UCC has superannuated.

References

- Abhyankar A V, Dash P K, Saxena P, Bhargava R, Parida M M, Jana A M, Sahni A K and Rao P V 2006 Comparison of a dipstick dot-ELISA with commercial assays for anti-dengue virus IgM antibodies; *Viral Immunol.* **19** 630–636
- Agarwal R, Chaturvedi U C, Misra A, Mukerjee R, Kapoor S, Nagar R, Tandon R and Mathur A 1998 Production of cytotoxic factor by peripheral blood mononuclear cells (PBMC) in patients with dengue haemorrhagic fever; *Clin Exp Immunol.* **112** 340–344
- Agarwal R, Kapoor S, Nagar R, Misra A, Tandon R, Mathur A, Misra A K, Srivastava K L and Chaturvedi U C 1999a A clinical study of the patients with dengue haemorrhagic fever during the epidemic of 1996 at Lucknow, India, *Southeast Asian J. Trop. Med. Publ. Hlth.* **30** 735–740
- Agarwal R, Elbishbishi E A, Chaturvedi U C, Nagar R and Mustafa A S 1999b Profile of transforming growth factor-beta1 in patients with dengue haemorrhagic fever; *Int. J. Exp. Pathol.* **80** 143–149
- Anandaramo R, Swaminathan S, Fernando S, Jana A M and Khanna N 2005 A custom-designed recombinant multi-epitope protein as a dengue diagnostic reagent; *Protein Exp. Purif.* **41** 136–147
- Anandaramo R, Swaminathan S, Fernando S, Jana A M and Khanna N 2006 Recombinant multi-epitope protein for early detection of dengue infections; *Clin. Vaccine Immunol.* **13** 59–67
- Angel B and Joshi V 2008 Distribution and seasonality of vertically transmitted dengue viruses in Aedes mosquitoes in arid and semi-arid areas of Rajasthan, India; *J. Vector Borne Dis.* **45** 56–59
- Arunachalam N, Murty U S, Kabilan L, Balasubramanian A, Thenmozhi V, Narahari D, Ravi A and Satyanarayana K 2004 Studies on dengue in rural areas of Kurnool District, Andhra Pradesh, India; *J. Am. Mosq. Control Assoc.* **20** 87–90
- Balaya S, Paul S D, D’Lima L V and Pavri K M 1969 Investigations on an outbreak of dengue in Delhi in 1967; *Indian J Med Res.* **57** 767–774
- Basu A, Jain P, Gangodkar S V, Shetty S and Ghosh K 2008 Dengue 2 virus inhibits in vitro megakaryocytic colony formation and induces apoptosis in thrombopoietin-inducible megakaryocytic differentiation from cord blood CD34+ cells; *FEMS Immunol. Med. Microbiol.* **53** 46–51
- Basu A and Chaturvedi U C 2008 Vascular endothelium: the battle field of dengue viruses; *FEMS Immunol. Med. Microbiol.* **53** 287–299
- Bharaj P, Chahar H S, Pandey A, Diddi K, Dar L, Guleria R, Kabra S K and Broor S 2008 Concurrent infections by all four dengue virus serotypes during an outbreak of dengue in 2006 in Delhi, India; *Viol. J.* **9** 5–10
- Bhargava A, Chaturvedi U C, Srivastava N and Mathur A 1990 Dengue virus-induced suppressor factor has two disulphide-bonded chains which bears anti-idiotypic and I-A and I-J determinants; *Curr. Sci.* **58** 157–160
- Bhattacharjee N, Mukherjee K K, Chakravarti S K, Mukherjee M K, De P N, Sengupta M, Banik G B, Bhowmick P, Sinha S K and Chakraborty M S 1993 Dengue haemorrhagic fever (DHF) outbreak in Calcutta—1990; *J. Commun. Dis.* **25** 10–14

- Carey D E, Myers R M, Reuben R and Rodrigues F M 1966 Studies on dengue in Vellore, South India; *Am. J. Trop. Med. Hyg.* **15** 580–587
- Chatterjee S N, Chakravarti S K, Mitra A C and Sarkar J K 1965 Virological investigation of cases with neurological complications during the outbreak of haemorrhagic fever in Calcutta; *J. Indian Med. Assoc.* **45** 314–316
- Chaturvedi U C, Mathur A, Kapoor A K, Mehrotra N K and Mehrotra R M. 1970a Virological study of an epidemic of febrile illness with haemorrhagic manifestations at Kanpur, India, during 1968; *Bull. World Health Organ.* **43** 289–293
- Chaturvedi U C, Kapoor A K, Mathur A, Chandra D, Khan A M and Mehrotra R M. 1970b A clinical and epidemiological study of an epidemic of febrile illness with haemorrhagic manifestations which occurred at Kanpur, India, in 1968; *Bull. World Health Organ.* **43** 281–287
- Chaturvedi U C, Mathur A, Kapoor A K, Tandon H O and Mehrotra R M. 1972 Clinico-virological study of the recurrence of dengue epidemic with haemorrhagic manifestations at Kanpur during 1969; *Indian J. Med. Res.* **60** 329–233
- Chaturvedi U C, Mathur A, Kapoor A K, Agarwal S K, Tandon H O and Mehrotra R M. 1974 Clinico-virological study of an outbreak of dengue-like illness at Hardoi (U.P.); *Indian J. Med. Res.* **62** 827–830
- Chaturvedi U C 1984 Dengue virus-induced suppressor pathway; *Curr. Sci.* **53** 971–976
- Chaturvedi U C, Pahwa M and Mathur A 1987 Dengue virus-induced helper T cells; *Indian J. Med. Res.* **86** 1–8
- Chaturvedi U C, Dhawan R, Khanna M and Mathur A 1991a Breakdown of the blood-brain barrier during dengue virus infection of mice; *J. Gen. Virol.* **72** 859–866
- Chaturvedi P, Mukherjee R, Chaturvedi U C and Mathur A 1991b Dengue virus-induced helper cytokine has two polypeptide chains which bear different determinants; *Int. J. Exp. Pathol.* **72** 665–672
- Chaturvedi P, Chaturvedi U C and Mukherjee R. 1992b Transmission of dengue virus-induced helper signal to B cell via macrophages; *Int. J. Exp. Pathol.* **73** 773–782
- Chaturvedi P, Mukherjee R, Chaturvedi U C and Mathur A. 1992a Characterization of the dengue virus-induced helper cytokine; *Int. J. Exp. Pathol.* **73** 263–272
- Chaturvedi U C, Mukerjee, R and Dhawan R 1994 Active immunization by a dengue virus-induced cytokine; *Clin. Exp. Immunol.* **96** 202–207
- Chaturvedi U C, Dhawan R and Mukerjee R 1997 Immunosuppression and cytotoxicity of dengue infection in the mouse model; in *Dengue and dengue haemorrhagic fever* (eds) D J Gubler and G Kuno (Wallingford, Oxon, UK: CAB International Press) pp 291–312
- Chaturvedi U C, Raghupathy R, Pacsa A S, Elbishbishi E A, Agarwal R, Nagar R, Misra A, Kapoor S, Mathur A, Khan M A Y and Azizieh F 1999a Shift from a Th1-Type Response to Th2-Type in Dengue Haemorrhagic Fever; *Curr. Sci.* **76** 63–69
- Chaturvedi U C, Agarwal R, Misra A, Mukerjee R, Kapoor S and Nagar R 1999b Cytotoxic factor in dengue haemorrhagic fever; *Med. Principles Pract.* **8** 26–31
- Chaturvedi U C, Elbishbishi E A, Agarwal R, Raghupathy R, Nagar R, Tandon R, Younis O I and Azizieh F 1990c Sequential production of cytokines by dengue virus infected human peripheral blood leukocyte cultures; *J. Med. Virol.* **59** 335–340
- Chaturvedi U C, Agarwal R, Elbishbishi E A and Mustafa A S 2000 Cytokine Cascade in Dengue Haemorrhagic Fever: Implications for Pathogenesis; *FEMS Immunol. Med. Microbiol.* **28** 183–188
- Chaturvedi U C, Elbishbishi E A, Agarwal R and Mustafa A S. 2001 Cytotoxic factor-autoantibodies: possible role in the pathogenesis of dengue haemorrhagic fever; *FEMS Immunol. Med. Microbiol.* **30** 181–186
- Chaturvedi U C and Shrivastava R 2004 Dengue Haemorrhagic Fever: A Global Challenge; *Indian J. Med. Microbiol.* **22** 5–6
- Chaturvedi U C, Shrivastava R and Nagar R. 2005 Dengue vaccines: problems and prospects; *Indian J. Med. Res.* **121** 639–652
- Chaturvedi U C, Nagar R and Shrivastava R. 2006a Macrophage and dengue virus: friend or foe?; *Indian J. Med. Res.* **124** 23–40
- Chaturvedi U C, Nagar R and Shrivastava R 2006b Dengue and dengue haemorrhagic fever: implications of host genetics; *FEMS Immunol. Med. Microbiol.* **47** 155–166
- Chaturvedi U C, Shrivastava R, Tripathi R K and Nagar R 2007 Dengue virus-specific suppressor T cells: current perspectives; *FEMS Immunol. Med. Microbiol.* **50** 285–299
- Chouhan G S, Rodrigues F M, Shaikh B H, Ilkal M A, Khangar S S, Mathur K N, Joshi K R and Vaidhye N K 1990 Clinical & virological study of dengue fever outbreak in Jalore city, Rajasthan 1985; *Indian J. Med. Res.* **91** 414–418
- Cherian T, Ponnuraj E, Kuruvilla T, Kirubakaran C, John T J and Raghupathy P 1994 An epidemic of dengue haemorrhagic fever & dengue shock syndrome in & around Vellore; *Indian J. Med. Res.* **100** 51–56
- Dash P K, Parida M M, Saxena P, Kumar M, Rai A, Pasha S T and Jana A M. 2004 Emergence and continued circulation of dengue-2 (genotype IV) virus strains in northern India; *J. Med. Virol.* **74** 314–322
- Dash P K, Saxena P, Abhyankar A, Bhargava R and Jana A M 2005 Emergence of dengue virus type-3 in northern India; *Southeast Asian J. Trop. Med. Public Health* **36** 370–377
- Dash P K, Parida M M, Saxena P, Abhyankar A, Singh C P, Tewari K N, Jana A M, Sekhar K and Rao P V 2006 Reemergence of dengue virus type-3 (subtype-III) in India: implications for increased incidence of DHF & DSS; *Virol. J.* **6** 3–55
- Dar L, Broor S, Sengupta S, Xess I and Seth P 1999 The first major outbreak of dengue hemorrhagic fever in Delhi, India; *Emerg. Infect. Dis.* **5** 589–590
- Dar L, Gupta E, Narang P and Broor S 2003 Co-circulation of dengue serotypes, Delhi, India, 2003; Letter to the editor. *Emerg. Infect. Dis.* **12** 352–353
- Garg P, Nagpal J, Khairnar P and Seneviratne S L 2008 Economic burden of dengue infections in India; *Trans. R. Soc. Trop. Med. Hyg.* **102** 570–577
- Ghosh S N, Pavri K M, Singh K R, Sheikh B H, D'lima L V, Mahadev P V and Ramachandra Rao T 1974 Investigations on the outbreak of dengue fever in Ajmer City, Rajasthan State in 1969. Part I. Epidemiological, clinical and virological study of the epidemic; *Indian J. Med. Res.* **62** 511–522

- Ghosh K, Gangodkar S, Jain P, Shetty S, Ramjee S, Poddar P and Basu A. 2008 Imaging the interaction between dengue 2 virus and human blood platelets using atomic force and electron microscopy; *J. Electron Microsc. (Tokyo)* **57** 113–118
- Gubler D J. 1997 Dengue/dengue haemorrhagic fever: its history and resurgence as a global public health problem; in *Dengue and dengue haemorrhagic fever* (eds) D J Gubler and G Kuno (Wallingford, Oxon, UK: CAB International Press) pp 1–22
- Gulati S and Maheshwari A 2007 Atypical manifestations of dengue; *Trop. Med. Int. Health* **12** 1087–1095
- Gupta E, Dar L, Kapoor G and Broor S 2006 The changing epidemiology of dengue in Delhi, India; *Viol. J.* **3** 92
- Halstead S B 1970 Observations related to pathogenesis of dengue hemorrhagic fever. VI. Hypotheses and discussion; *Yale J. Biol. Med.* **42** 350–362
- Halstead S B 2002 Dengue; *Curr. Opin. Infect. Dis.* **15** 471–476
- Halstead S B 2007 Dengue; *Lancet* **370** 1644–1652
- Hapugoda M D, Batra G, Abeyewickreme W, Swaminathan S and Khanna N 2007 Single antigen detects both immunoglobulin M (IgM) and IgG antibodies elicited by all four dengue virus serotypes; *Clin. Vaccine Immunol.* **14** 1505–1514
- Jaiswal S, Khanna N and Swaminathan S 2004 High-level expression and one-step purification of recombinant dengue virus type 2 envelope domain III protein in *Escherichia coli*; *Protein Exp. Purif.* **33** 80–91
- Kabra S K, Verma I C, Arora N K, Jain Y and Kalra V 1992 Dengue haemorrhagic fever in children in Delhi; *Bull. World Health Organ.* **70** 105–108
- Khanam S, Khanna N and Swaminathan S 2006 Induction of neutralizing antibodies and T cell responses by dengue virus type 2 envelope domain III encoded by plasmid and adenoviral vectors; *Vaccine.* **24** 6513–6525
- Khanam S, Rajendra P, Khanna N and Swaminathan S 2007 An adenovirus prime/plasmid boost strategy for induction of equipotent immune responses to two dengue virus serotypes; *BMC Biotechnol.* **15** 7–10
- Kimura R and Hotta S 1944 Studies on dengue fever (IV). On inoculation of dengue virus into mice; *Nippon Igaku No.* **3379** 629–633
- Kumar A, Sharma S K, Padbidri V S, Thakare J P, Jain D C and Datta K K 2001 An outbreak of dengue fever in rural areas of northern India; *J. Commun. Dis.* **33**:274–281
- Kumar R, Tripathi S, Tambe J J, Arora V, and Nag V L 2008 Dengue encephalopathy in children in Northern India: Clinical features and comparison with non dengue; *J. Neurol. Sci.* **269** 41–48
- Kukreti H, Chaudhary A, Rautela R S, Anand R, Mittal V, Chhabra M, Bhattacharya D, Lal S and Rai A 2008 Emergence of an independent lineage of dengue virus type 1 (DENV-1) and its co-circulation with predominant DENV-3 during the 2006 dengue fever outbreak in Delhi; *Int. J. Infect. Dis.* PMID: 18495513 [PubMed [Epub ahead of print]]
- Kurukumbi M, Wali J P, Broor S, Aggarwal P, Seth P, Handa R, Dhar L and Vajapayee M 2001 Seroepidemiology and active surveillance of dengue fever/dengue haemorrhagic fever in Delhi; *Indian J. Med. Sci.* **55** 149–156
- Mahadev P V, Kollali V V, Rawal M L, Pujara P K, Shaikh B H, Ilkal M A, Pathak V, Dhanda V, and Banerjee K 1993 Dengue in Gujarat state, India during 1988 & 1989; *Indian J. Med. Res.* **97** 135–144
- Mehendale S M, Risbud A R, Rao J A and Banerjee K 1991 Outbreak of dengue fever in rural areas of Parbhani district of Maharashtra (India); *Indian J. Med. Res.* **93** 6–11
- Mills K H G 2004 Regulatory T cells: Friend or foe in immunity to infection?; *Nat. Rev. Immunol.* **4** 841–855
- Misra A, Mukerjee, R and Chaturvedi, U C 1996a Production of nitrite by dengue virus-induced cytotoxic factor; *Clin. Exp. Immunol.* **104** 650–655
- Misra A, Mukerjee R and Chaturvedi U C 1996b Release of reactive oxygen intermediates by dengue virus-induced macrophage cytotoxin; *Int. J. Exp. Pathol.* **77** 237–242
- Misra A, Mukerjee R and Chaturvedi U C 1998 Respiratory burst by dengue virus-induced cytotoxic factor; *Med. Principles Pract.* **7** 251–260
- Mongkolsapaya J, Dejnirattisai W and Xu X N 2003 Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever; *Nat. Med.* **9** 921–927
- Mongkolsapaya J, Duangchinda T, Dejnirattisai W, Vasanawathana S, Avirutnan P, and Jairungsri A. 2006 T cell responses in dengue hemorrhagic fever: are cross-reactive T cells suboptimal?; *J. Immunol.* **176** 3821–3829
- Mosmann T R and Sad S 1996 The expanding universe of T cell subsets: Th1, Th2 and more; *Immunol. Today* **17** 138–146
- Mukherjee K K, Chakravarti S K, Dey P N, Dey S and Chakraborty M S 1987 Outbreak of febrile illness due to dengue virus type 3 in Calcutta during 1983; *Trans. R. Soc. Trop. Med. Hyg.* **81** 1008–1010
- Mukherjee R, Chaturvedi P and Chaturvedi U C 1993a Identification of a receptor on macrophages for the dengue virus-induced suppressor cytokine; *Clin. Exp. Immunol.* **91** 257–265
- Mukherjee R, Chaturvedi P and Chaturvedi, U C 1993b Binding of the two polypeptide chains of dengue virus-induced suppressor cytokine to its receptor isolated from macrophages; *Int. J. Exp. Pathol.* **74** 259–266
- Mukherjee R, Chaturvedi, P and Chaturvedi U C 1994 Specific receptor for dengue virus-induced suppressor cytokine on macrophages and lymphocytes; *Int. J. Exp. Pathol.* **75** 29–36
- Mukerjee R, Misra A and Chaturvedi U C 1996 Dengue virus-induced cytotoxin releases nitrite by spleen cells; *Int. J. Exp. Pathol.* **77** 45–51
- Mukerjee R, Chaturvedi U C, Vaughn D W and Kalayanarooj S 1997 Purification and pathogenicity of the cytotoxic factor from the cases of dengue haemorrhagic fever; *Curr. Sci.* **72** 494–501
- Myers R M and Carey D E 1967 Concurrent isolation from patient of two arboviruses, Chikungunya and dengue type 2; *Science.* **157** 1307–1308
- Myers R M, Carey D E, Banerjee K, Reuben R and Ramamurti D V 1968 Recovery of dengue type 3 virus from human serum and *Aedes aegypti* in South India; *Indian J. Med. Res.* **56** 781–787
- Myers R M, Carey D E, DeRanitz C M, Reuben R and Bennet B 1969 Virological investigations of the 1966 outbreak of Dengue type 3 in Vellore, Southern India; *Indian J. Med. Res.* **57** 1392–1401
- Myers R M, Varkey M J, Reuben R and Jesudass E S 1970 Dengue outbreak in Vellore, southern India, in 1968, with isolation of

- four dengue types from man and mosquitoes; *Indian J. Med. Res.* **58** 24–30
- Myers R M and Varkey M J 1971 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.* **59** 1231–1236
- Nagarkatti P S and Nagarkatti M 1983 Effect of experimental dengue virus infection on immune response of the host. I. Nature of changes in T suppressor cell activity regulating the B and T cell responses to heterologous antigens; *J. Gen. Virol.* **64** 1441–1447
- Padbidri V S, Thakre J P, Risbud A R, Joshi G D, Singh A, Mavale M S and Gupta V P 1996 An outbreak of dengue hemorrhagic fever in Jammu; *Indian J. Virol.* **12** 83–87
- Pattanaik P, Srivastava A, Abhyankar A, Dash P K, Parida M M and Lakshmana Rao P V 2006 Fusogenic peptide as diagnostic marker for detection of flaviviruses; *J. Postgrad. Med.* **52** 174–178
- Parida M M, Upadhyay C, Saxena P, Dash P K, Jana A M and Seth P 2001 Evaluation of a dipstick ELISA and a rapid immunochromatographic test for diagnosis of Dengue virus infection; *Acta Virol.* **45** 299–304
- Parida M M, Dash P K, Upadhyay C, Saxena P and Jana A M 2002 Serological and Virological investigation of an outbreak of dengue fever in Gwalior, India; *Indian J. Med. Res.* **116** 248–254
- Parida M M, Horioko K, Ishida H, Dash P K, Saxena P, Jana A. M, Islam M A, Inoue S, Hosaka N and Morita K 2005 Rapid detection and differentiation of dengue virus serotypes by a real-time reverse transcription-loop-mediated isothermal amplification assay; *J. Clin. Microbiol.* **43** 2895–2903
- Rigau-Perez J G, Clark G G, Gubler D J, Reiter P, Sanders E J and Vorndam A V 1998 Dengue and dengue hemorrhagic fever; *Lancet* **352** 971–977
- Rizvi N, Chaturvedi P and Chaturvedi U C 1993 Binding of macrophages and B lymphocytes mediated by dengue virus antigen and the virus-induced helper cytokine; *Int. J. Exp. Pathol.* **74** 187–194
- Rodrigues F M, Pavri K M, Dandawate C N, Banerjee K and Bhatt P N 1973 An investigation of the aetiology of the 1966 outbreak of febrile illness in Jabalpur, Madhya Pradesh; *Indian J. Med. Res.* **61** 1462–1470
- Sabin A B and Schlesinger M C 1945 Production of immunity to dengue with virus modified by propagation in mice; *Science* **101** 640–642
- Sarkar J K, Pavri K M, Chatterjee S N, Chakravarty S K and Anderson C R 1964 Virological and serological studies of cases of haemorrhagic fever in Calcutta; *Indian J. Med. Res.* **52** 684–691
- Saxena P, Dash P K, Santhosh S R, Srivastava A, Parida M M and Rao P V L 2008 Development and evaluation of one step single tube multiplex RT-PCR for rapid detection and typing of dengue viruses; *Virol. J.* **5** 20
- Saxena P, Parida M M, Dash P K, Santhosh S R, Shrivastava A, Tripathi N K, Gupta N, Sahani A K, Bhargava R, Singh C P, Tewari, K N, Sekhar K and Rao P V L 2006 Co-Circulation of Dengue Virus Serotypes in Delhi, India, 2005: Implication for Increased DHF/DSS; *Dengue Bull.* **30** 283–287
- Shah I, Deshpande G C and Tardeja P N. 2004 Outbreak of dengue in Mumbai and predictive markers for dengue shock syndrome; *J. Trop. Pediatr.* **50** 301–305
- Shrivastava R, Srivastava S, Upreti R K and Chaturvedi U C 2005 Effects of dengue virus infection on peripheral blood cells of mice exposed to hexavalent chromium with drinking water; *Indian J. Med. Res.* **122** 111–119
- Shrivastava R, Nagar R, Ravishankar G A, Upreti R K and Chaturvedi U C 2007 Effect of pretreatment with chromium picolinate on haematological parameters during dengue virus infection in mice; *Indian J. Med. Res.* **126** 440–446
- Shukla M I and Chaturvedi U C 1981 Dengue virus-induced suppressor factor stimulates production of prostaglandin to mediate suppression; *J. Gen. Virol.* **66** 241–249
- Shukla M I and Chaturvedi U C 1983 Transmission of dengue virus-induced suppressor signal from macrophage to lymphocyte occurs by cell contact; *Br. J. Exp. Pathol.* **64** 87–92
- Shukla M I and Chaturvedi U C 1982 In vivo role of macrophages in transmission of dengue virus-induced suppressor signal to T lymphocytes; *Br. J. Exp. Pathol.* **63** 522–530
- Sierra B, Garcia G, Perez A B, Morier L, Rodriguez R, Alvarez M and Guzman M G 2002 Long-term memory cellular immune response to dengue virus after a natural primary infection; *Int. J. Infect. Dis.* **6** 125–128
- Singh J, Balakrishnan N, Bhardwaj M, Amuthadevi P, George E G, Subramani K, Soundararajan K, Appavoo N C, Jain D C, Ichhpujani R L, Bhatia R. and Sokhey J. 2000 Silent spread of dengue and dengue haemorrhagic fever to Coimbatore and Erode districts in Tamil Nadu, India, 1998: need for effective surveillance to monitor and control the disease; *Epidemiol. Infect.* **125** 195–200
- Singh K R and Paul S D 1969 Isolation of dengue viruses in *Aedes albopictus* cell cultures; *Bull. World Health Organ.* **40** 982–998
- Singh U B, Maitra A, Broor S, Rai A, Pasha S T and Seth P 1999 Partial nucleotide sequencing and molecular evolution of epidemic causing Dengue 2 strains; *J. Infect. Dis.* **180** 959–965
- Soundravally R and Hoti S L 2007a Immunopathogenesis of dengue hemorrhagic fever and shock syndrome: role of TAP and HPA gene polymorphism; *Hum. Immunol.* **68** 973–979
- Soundravally R and Hoti S L 2008 Significance of Transporter Associated with Antigen Processing 2 (TAP2) Gene Polymorphisms in Susceptibility to Dengue Viral Infection; *J. Clin. Immunol.* **28** 256–262
- Soundravally R, Sankar P, Hoti S L, Selvaraj N, Bobby Z and Sridhar M G 2008 Oxidative stress induced changes in plasma protein can be a predictor of imminent severe dengue infection; *Acta Trop.* **106** 156–161
- Tai D F, Lin C Y, Wu T Z, Huang J H and Shu P Y 2006 Artificial receptors in serologic tests for the early diagnosis of dengue virus infection; *Clin. Chem.* **52** 1486–1491
- Tandon P, Chaturvedi U C and Mathur A 1979 Dengue virus-induced thymus-derived suppressor cells in the spleen of mice; *Immunology* **38** 653–658
- Tewari S C, Thenmozhi V, Katholi C R, Manavalan R, Munirathinam A and Gajanana A 2004 Dengue vector prevalence and virus

- infection in a rural area in south India; *Trop. Med. Int. Health.* **9** 499–507
- Tripathi N K, Shrivastva A, Pattnaik P, Parida M M, Dash P K, Gupta N, Jana A M and Rao P V L 2007a. Production of IgM specific recombinant dengue multiepitope protein for early diagnosis of dengue infection; *Biotechnol. Prog.* **23** 488–493
- Tripathi N K, Shrivastva A, Pattnaik P, Parida M M, Dash P K, Jana A M and Rao P V L 2007b Production, purification and characterization of recombinant dengue multiepitope protein; *Biotechnol. Appl. Biochem.* **46** 105–113
- Tripathi R K, Khare M and Chaturvedi U C 1997 Internalization of dengue virus- induced suppressor cytokine during transmission of the suppressor signal via macrophage; *Indian J. Exp. Biol.* **35** 850–854
- World Health Organization 1997 Clinical diagnosis; in *Dengue haemorrhagic fever: Diagnosis, treatment, prevention and control* 2nd edition (Geneva:WHO) pp 12–23

ePublication: 15 October 2008