Review Article

Indian J Med Res 124, July 2006, pp 23-40

Macrophage & dengue virus: Friend or foe?

U.C. Chaturvedi*, Rachna Nagar & Richa Shrivastava**

Department of Microbiology, K.G. Medical University, Lucknow, India

Received December 12, 2005

The cells of monocyte-macrophage (Mϕ) lineage play important roles both in innate and adaptive immune responses. They are the first line of defence in body and their job is to phagocytose a foreign invader, the pathogen, digest it and remove it. Mϕ help body in mounting the antigen-specific immune response by presenting the digested pathogen antigen in conjunction with major histocompatibility complex (MHC) class II molecules to recruit B and T lymphocytes response. Usually Mϕ succeed in their job of eliminating most pathogens from the body but sometimes the pathogen strikes a "friendship" with them and starts using them for its benefit. A number of pathogens, including dengue virus (DV), subvert Mϕ and use them for their replication, increasing the severity of damage to the body. This duality may be related to the fact that Mϕ serve as efficient host cell for DV replication, in addition to being responsible for innate immunity and for initiating adaptive immune responses. This review gives a brief overview of the various roles of Mϕ (enmity and friendship) during dengue virus infection.

Key words Cytokines - cytokine receptors - dendritic cell - dengue virus - DHF - Langerhan's cell - Kupffer's cell - macrophage - pathogenesis - signal transmission

Dengue is a mosquito-borne virus infection, found in tropical and sub-tropical regions around the world, predominantly in urban and semi-urban, and now in rural areas also. Dengue is caused by four distinct viruses (serotypes 1 to 4) that are closely related antigenically. Humans are the main amplifying host of the virus. Recovery from infection by one serotype provides long lasting immunity against that serotype but confers only partial and transient protection against subsequent infection by the other three. It has been suggested that sequential infection increases the risk of more serious disease resulting in dengue haemorrhagic fever (DHF). The prevalence of dengue has grown dramatically in recent decades. The disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, Southeast Asia and the Western Pacific. Southeast Asia and the Western Pacific are most seriously affected. Some 2500 million people, two fifths of the world's population are now at risk from dengue. As per WHO's current estimates there may be 50 million cases of dengue infection worldwide every

Present address: *201-Annapurna Apartments, No. 1, Bishop Rocky Street, Faizabad Road, Lucknow 226007, India
**Central Food Technology Research Institute, Mysore, India

year. During epidemics of dengue, attack rates among susceptibles are 40 to 90 per cent. An estimated 500,000 cases of DHF require hospitalization each year, of whom a very large proportion are children[1-3]. DHF has been classified into four grades on the basis of the clinical presentation and laboratory findings; the mildest is grade I and the most severe is grade IV. The characteristic features of DHF are increased capillary permeability without morphological damage to the capillary endothelium, altered number and functions of leucocytes, increased haematocrit and thrombocytopaenia. Extensive plasma leakage in various serous cavities of the body including the pleura, pericardium and peritoneal cavities in DHF grades III and IV may result in profound shock, the dengue shock syndrome (DSS). Today DHF affects most Asian countries and has become a leading cause of hospitalization and death among children in several of them. At present, there is no specific therapy available for DHF. Appropriate symptomatic treatment has been successful in reducing the mortality of DHF[4-6].

Macrophages are part of the innate immune system and are derived from monocytes which grow in the bone marrow. They enter the bloodstream, circulate all over the body and squeeze through the endothelium into tissues. Once in the tissues, they are called macrophages. Some monocytes differentiate into specialized cells such as dendritic cells (DC), Langerhans cells (LC), Kupffer's cells (KC) or microglia, *etc.* (Table I). The term 'Mϕ' has been used for this group of cells unless specified. The main role of these cells is in providing the first line of defence as innate immune response. Mϕ ingest the pathogens, digest them and present their antigens with major histocompatibility complex (MHC) class II molecules on their cell membranes to B lymphocytes and T cells to generate antigen-specific immune response. Mϕ again come into play as they opsonize the virus or the cells with antibodies attached to control and eleminate the virus. Mostly Mϕ succeed in their job of eliminating a pathogen from body but sometimes the pathogen strikes a friendship with them and starts using them for its benefit. A number of pathogens, including dengue virus (DV), subvert Mϕ and use them for their replication increasing severity of the damage to body[8,17,18]. The extent of DV replication during the early periods of infection may determine clinical outcomes, that may be from asymptomatic infection to febrile illness, dengue fever (DF), to life-threatening disease, DHF/DSS. The impact of DV infection on innate immunity may be the determining factor. The cells of macrophage-lineage, interstitial DC and LC constitute the first line of the innate host defense against invading DV in skin where it replicates after the initial bite by infected mosquito. Early activation of natural killer (NK) cells and type-I interferon-dependent immunity may limit viral replication at the early stages of DV infection. The ability of infecting DV to counter the innate antiviral immunity might account for differences in clinical outcome and the virulence observed between different viral strains. This review gives a brief overview of the various roles of macrophages (enmity and friendship) during DV infection.

Macrophage

Macrophages the "big eater" cells, are found in tissues and are responsible for phagocytosis of pathogens, dead cells and cellular debris. Mϕ are large cells with a round or indented nucleus, a well-developed Golgi apparatus, abundant endocytotic vacuoles, lysosomes, and phagolysosomes, and a plasma membrane covered with ruffles or microvilli. Activation alters the morphology and functional activity of Mϕ so that they become avidly phagocytic. It is initiated by cytokines, such as the Mϕ activation factor (maf) and the Mϕ migration-inhibitory factor (mmif), immune complexes, c3b, and various peptides, polysaccharides, and immunologic adjuvants.The Mϕ colony-stimulating factor is a glycoprotein growth factor that causes the committed cell line to proliferate and mature into Mϕ. Due to their role in phagocytosis, Mϕ are involved in many diseases of the immune system, participating in the formation of inflammatory lesions. When fighting viruses, Mϕ are dispatched to the site. However, until the killer T cells for the virus are formed, the Mϕ do

more damage than help. They not only destroy cells infected with the virus, but also destroy several surrounding non-infected cells[8,17,18].

Macrophage functions during DV infection

During DV infection in mice the number of Mϕ is reduced in the spleen and peritoneal cavity and several functions are depressed. These include depressed phagocytic activity and reduced migration on a glass surface of splenic and peritoneal-cavity Mϕ[19]. Fc-receptor-mediated attachment and ingestion of opsonized sheep erythrocytes (EA) by the Mϕ of spleen and peritoneal cavity are also adversely affected during DV infection in mice. A loss in the capacity to attach and ingest EA is noted, the lowest values of attachment index (AI) and phagocytic index (PI) being reached on day 4. At later periods the AI values increase but continue to be significantly less than those in uninfected control mice. The PI values continue to be lower throughout. The dichotomy between the Fc-mediated attachment and ingestion may be a mechanism for prevention of virus infection of Mϕ[8]. DV infection induces production of a cytokine, the cytotoxic factor (CF). Many defects in Mϕ functions in DV infection have been shown to be mediated by production of the virus-induced CF. Mice given CF intravenously show a rapid fall in total numbers of peritoneal and spleen cells. The number of cells in the peritoneal cavity recover in 48 h but recovery in the spleen is not significant. The capacity of the splenic and peritoneal Mϕ to attach and ingest opsonized sheep erythrocytes is significantly reduced, the lowest values of AI and PI being observed within 2-3 h. At later periods the AI values increase markedly but the PI values remain depressed. The effect is dose-dependent. The effect on Fc-receptor functions of Mϕ in DV-infected mice thus appears to be mediated through CF[20].

Role of macrophage in replication of DV

Mϕ are the principal cells to replicate DV[8]. The efficiency of DV replication by Mϕ is higher than that of peripheral B-lymphocytes but is lower than that of human lymphoblastoid cell lines. Mitogen-treated and untreated Mϕ replicate DV equally well. Unstimulated peripheral lymphocytes inoculated immediately after isolation adsorb DV but do not support its replication[21]. It has been suggested that DV can infect Mϕ through a trypsin-sensitive virus receptor or through a trypsin-resistant Fc receptor[22]. The first encounter of the virus

Table I. Characterstics of macrophage and its lineage cells*

	Macrophage cells	Dendritic cells	Langerhans cells	Microglia	Kupffer's cells
Phagocytosis	Yes	Yes	Yes	Yes	Yes
MHC II antigen	Yes	Yes	Yes	Yes	Yes
Fc-receptor	Yes	Yes	Yes	Yes	Yes
C3-receptor	Yes	No	Yes	Yes	?
Acid phosphatase	Yes	No	Yes	Yes	Yes
Esterase	Yes	No	Yes	No	Yes
ATPase	Yes	Yes	Yes	Yes	Yes
Endogenous peroxidase	Yes	No	No	No	Yes
CD14	Yes	Yes	Yes	Yes	Yes
Entry of DV	Yes	Yes	Yes	Yes	Yes
Production of DV	Yes	Yes	Yes	Not known	No
References#	8, 9	10,11	11, 12	13, 14	15, 16

*Modified from Chaturvedi *et al*[7]
#Only selected references are cited here
DV, Dengue virus; MHC, major histocompatibility complex

with the host may be through binding to attachment receptors, such as the C-type lectins DC- and L-SIGN, which may play an important role in infection with a large number of enveloped viruses by capturing, concentrating and transmitting infectious virions. Once a virus reaches its target cell, a cascade of events generally starting with the interaction of viral envelope glycoproteins with specific entry receptors and co-receptors is necessary in order to trigger the virus-cell membrane fusion. DV enters Mϕ through a virus receptor or the Fc-receptor as an immune complex. Schlesinger and Chapman[23] have reported that the FcγRI extracellular domain is sufficient for internalization of infectious DV immune complexes through a mechanism that does not involve classical immunoreceptor tyrosine-based activation motif-dependent signaling. Moreno-Altamirano *et al*[24] have described putative receptors for DV in primary cultures of human Mϕ while Reyes *et al*[25] have shown that heat shock protein 90 (HSP90) and HSP70 act as a receptor complex in human cell lines and in Mϕ. Further, both HSPs are associated with membrane microdomains in response to DV infection and cholesterol-rich membrane fractions are important in DV entry. Marovich *et al*[10] have shown that immature DC are most permissive for DV infection and may be early targets for infection. Human skin DC and LC are permissive for DV infection but blood-derived DC are 10-fold more permissive for DV infection than monocytes or macrophages[12]. DV effectively penetrates KC, but the infection does not result in any viral progeny[15]. The interstitial CD14+ cells in skin are permissive to DV and may contribute to an antiviral immune response[9]. DV can infect and persist in human haematopoietic cells and alter their proliferative capacity[26]. Pryor *et al*[27] studied the association of disease severity with replication of DV isolates from Asia or America in Mϕ and also the constructed recombinant DV with substitutions at residue 390 in the envelope glycoprotein (E390). The American strain does not replicate as well as the two Asian strains. For the recombinant viruses, substitution of Asn (Asian) at E390 with Asp (American) results in decreased ability to replicate in Mϕ. This indicates that the lack of association of native American DV-2 strains with severe disease is linked to reduced ability to replicate in Mϕ, and that Asp at E390 may contribute to this reduction. Using human Mϕ and DC, it has been demonstrated that the chimeric DV containing the E mutation has a lower virus output compared to the parental infectious clone. A larger reduction in virus output is observed for the triple mutant and the wild type, American genotype virus from which chimeric inserts are derived. It appears that the three changes function synergistically, although the E mutation alone gives a lower output compared to the 5'- and 3'-terminal mutations[28]. These changes may be responsible for decreased DV replication in human target cells and for virulence characteristics during infection. Ultrastructural analysis of early interaction of DV and Mϕ shows cell apoptosis and absence of DV replication that may abort infection[29]. Ho *et al*[30] have examined the effects of interferon (IFN)-α and IFN-γ in DV infection of DC and have shown that the pre-infection treatment with either IFN-α or IFN-γ effectively arms DC and limit viral production in infected cells. After infection, DV develops mechanisms to counteract the protection from lately added IFN-α, but not IFN-γ. This correlates with downregulated tyrosine-phosphorylation and DNA-binding activities of STAT1 and STAT3 transcription factors by DV. Moreover, DV infection by itself can activate STAT1 and STAT3 through IFN-α-dependent and both IFN-α-dependent and IFN-α-independent mechanisms, respectively[30]. Shresta *et al*[31] have demonstrated that IFNR-dependent control of primary DV infection involves both STAT1-dependent and STAT1-independent mechanisms. The STAT1 pathway is necessary for clearing the initial viral load, whereas the STAT1-independent pathway controls later viral burden and prevents dengue disease in mice. The STAT1-independent responses in mice with primary DV infection include the early activation of B and NK cells as well as the upregulation of MHC class I molecules on Mϕ and DV.

Antibody-dependent enhancement of DV replication

Halstead and colleagues were the first to suggest an association of increased risk for DHF

with a secondary DV infection[32-34]. This hypothesis has been supported in several studies with DV outbreaks in Southeast Asia and Cuba[35-37]. Passive transfer of antibody against DV increases virus titres in nonhuman primates[38]. In epidemic areas where DHF is associated with prior circulation of low level monotypic antibody, severe dengue disease could represent antibody-dependent enhancement (ADE) of infection of human Mϕ[39]. A positive correlation between peak DV titres and disease severity in humans has been demonstrated[40,41] supporting the *in vivo* importance of ADE. This supports the hypothesis that the severity of dengue in humans is regulated by non-neutralizing antibody[38]. The phenomenon of ADE of viral replication is not unique to DV, and may have far wider relevance in other viral infections also[42] . DV production is enhanced in cultures of Mϕ pretreated with phytohaemagglutinin (PHA) or bacterial lipopolysaccharide (LPS) while treatment with concanavalin A has little effect[43]. Enhancement of DV infection has been reported by treatment of a mouse Mϕ cell line Mk1 with pokeweed mitogen either before or during but not after virus inoculation. The infection enhancement is primarily due to an increase in the number of DV-infected cells but not to increased virus production in a cell[44]. Lipophilic derivatives of muramyl peptides have similar effect[45]. On the other hand, Chen *et al*[46] have reported that LPS markedly suppresses DV infection of primary human Mϕ when it is added to cultures prior to or together with, but not after, viral adsorption. It is suggested that LPS blocks DV entry into human Mϕ via its receptor CD14 and that a CD14-associated cell surface structure may be essential for the initiation of a DV infection[46]. Treatment of Mϕ with carrageenan, a specific Mϕ blocking agent, markedly suppresses DV production[43]. Nitric oxide also inhibits DV replication in mouse neuroblastoma cells in a dose and a multiplicity of infection dependent manner. The mechanism of inhibition is suppression of the RNA production, which correlates to production of the infectious particles[47].

Presentation of DV-antigen by macrophage

DV-infected Mϕ present DV antigen to B cells *in vitro* and *in vivo*, leading to their clonal expansion as shown by counting the virus-specific IgM antibody plaque-forming cells (PFC). The PFC response depends upon the number of DV-infected Mϕ. Superimposition of a heterologous antigen (Coxsackie B4 virus; CoxB) in a Mackaness type of experiment (simultaneous stimulation by two antigens) depresses the capacity of Mϕ to present both the homologous as well as heterologous antigen[48]. Live Mϕ are obligatory for DV antigen presentation to B-lymphocytes. Heat-killed or glutaraldehyde-fixed Mϕ do not present the DV antigen. Pre-treatment of Mϕ with the lysosomotropic compounds, ammonium chloride and chloroquine inhibit the antigen presentation. It is shown that even for presentation to B cells the DV antigen must be processed by Mϕ by a trypsin-like protease[49]. The results have also identified the serine group of proteases as the main enzymes involved in processing the DV antigen in Mϕ[50]. Further, if given simultaneously, the competition between DV and CoxB for antigen presentation to B cells occurs in Mϕ at the level of antigen processing[51].

In the natural infection, DV is introduced into human skin by an infected mosquito vector where it is believed to target immature DC and LC. On intradermal (i.d.) injection of DV, LC increase in numbers within 24 h at the site of injection. Subsequent re-challenge by the i.d. route produces an even more rapid serological response. A significant sharp drop in LC densities in the early post-injection phase directly correlates with the increased numbers of DC in the superficial dermis and interfollicular sinuses of draining lymph nodes[52]. Further, the appearance of endosomes in LC highlights the mode of antigen processing by the endocytic pathway[53]. DC plays a central role as major targets of DV infections and initiators of antiviral immune responses acquiring the capacity to promote cell-mediated immunity. However, separate evaluations of the maturation profiles of infected and

uninfected bystander cells show that infection impairs the ability of DC to upregulate cell surface expression of co-stimulatory, maturation, and MHC molecules, resulting in reduced T-cell stimulatory capacity. Infected DC does not respond to TNF-α as an additional maturation stimulus and are apoptotic. Interleukin-10 (IL-10) is detected in supernatants from cultures of DV-infected DC and co-cultures of DC and T cells[54]. This indicates an immune evasion strategy used by DV that directly impairs antigen-presenting cell function by maturation blockade and induction of apoptosis. Lozach *et al*[55] have reported that the interactions between DV E protein and the C-type lectin DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) are essential for DV infection of DC. Binding of mannosylated N-glycans on DV E protein to DC-SIGN triggers a rapid and efficient internalization of the viral glycoprotein. They observed that endocytosis-defective DC-SIGN molecules allow efficient DV replication, indicating that DC-SIGN endocytosis is dispensable for the internalization step in DV entry. This indicates a mechanism by which DC-SIGN enhances DV entry and infection.

Cytokine production by macrophage

Cytokines are low molecular weight, soluble proteins that are produced in response to an antigen and function as chemical messengers for regulating the innate and adaptive immune systems. They are produced by virtually all cells involved in innate and adaptive immunity. The activation of cytokine-producing cells triggers them to synthesize and secrete their cytokines. The cytokines, in turn, are then able to bind to specific cytokine receptors on other cells of the immune system and influence their activity in some manner. Cytokines are pleiotropic, redundant, and multifunctional and two different cytokines may be antagonistic or synergistic in their function. Cytokines that are produced primarily by macrophages regulate innate immunity. These are produced primarily in response to pathogen-associated molecules and mostly act on leucocytes and the endothelial cells to promote and control early inflammatory responses. The cytokines include tumour necrosis factor (TNF), IL-1, IL-6, IL-10, IL-12, IL-15, IL-18, interferon-alpha (IFN-α), IFN-β, transforming growth factor-beta (TGF-β) and chemokines like IL-8, macrophage inflammatory protein (MIP)-1α, MIP-1β, MCP-1, MCP-2, MCP-3, RANTES, *etc*[56-59].

Cytokines produced by macrophages in patients with dengue

The most significant finding reported for the first time on patients with DHF during 1996 was the shift from the predominant helper T cell type 1 (Th1) response observed in cases of DF to the Th2-type in severe cases of DHF grade IV. As the severity of the illness increases the response shifts to Th2-type in

Table II. Cytokines secreted by macrophages in patients with dengue

Cytokine	Dengue fever	Dengue haemorrhagic fever	References
Interleukin-1β	No change	No change	60-63
Interleukin-6	Increased	Marked increase	56, 58, 60, 64-66
Interleukin-8	Decreased	Marked increase	58, 66, 67
Interleukin-10	Decreased	Marked increase	56,58, 68
Interleukin-12	Marked increase	Decreased	58, 69
Interleukin-18	Increased	Marked increase	58, 70, 71
Tumour necrosis factor-α	Marked increase	Marked increase	56, 58, 60-63, 65, 72
Transforming growth factor-β	Decreased	Marked increase	58, 63, 73
Cytotoxic Factor-2	Increased	Marked increase	58, 59, 74-76
Macrophage inflammatory protein-1α,-1β	Present	Present	77

Other cytokines that are important in dengue disease are IL-2, IL-4, IL-13, IFN-γ and cytotoxic factor but are not produced by macrophages

majority of the cases with DHF grade IV[56]. Most of these cytokines are secreted by Mϕ in patients with dengue disease (Table II). IL-12 has a profound effect on the upregulation of Th1 cells while its absence shifts the balance towards Th2-type cytokines. IL-12 has been associated with clearance of virus, host recovery and protection in a large number of viral infections[78]. Elevated levels of IL-12 are seen in the patients with milder dengue illness (DF) and complete absence in the patients with DHF grades III and IV[69]. Thus, IL-12 may play a role in preventing the severe dengue disease by maintaining the Th1-type response. If this is true, IL-12 therapy may have profound beneficial effect on the outcome of severe dengue disease[58]. Increased levels of IL-8 in the sera and IL-8-mRNA in the peripheral blood mononuclear cells (PBMC) are associated with the increasing severity of DHF and death of patients. It has been suggested that presence of high levels of IL-8 may be a useful indicator of serious outcome of the dengue illness[67]. Further, the severity of disease and the duration of illness are correlated with the levels of TGF-β1, *i.e.*, the maximum levels of TGF-β1 are detected in patients with DHF grade IV[73]. Serum IL-6 concentrations are higher in patients with DHF and dengue shock syndrome[56,79]. IL-6 is produced mainly by mast cells and endothelial cells. It is an endogenous pyrogen that also increases endothelial cell permeability. Endothelial cells also produced IL-8, having potent proinflammatory and chemoattractant activity[80]. Activated neutrophils release proteinases such as elastase, which may facilitate neutrophil mediated endothelial injury, and activate the complement, coagulation, and fibrinolytic systems. Since increased levels of serum IL-8 and elastase are found in patients with severe infections, they may have an important role in pathogenesis of dengue infections[67,79].

Cytokine production during DV infection *in vitro*

After DV infection, the *in vitro*-differentiated macrophages secrete multiple innate cytokines and chemokines, including TNF-α, IFN-α, IL-1 β, IL-6, IL-8, IL-12, MIP-1α, and RANTES. DV induces a predominant Th1-type cytokine response during the first three days of infection of human PBMC cultures that is replaced by a Th2-type response later[57]. Medin *et al*[81] have indicated a role for the DV NS5 protein in the induction of IL-8 by DV infection. In addition, DV replication in Mϕ is enhanced and prolonged in the presence of LPS, and LPS-mediated synergistic production of IFN-α is seen[82]. The LPS-mediated enhancement of virus replication and synergistic IFN-α production suggests that concurrent bacterial infection may modulate cytokine-mediated disease progression during DV infection. DV infection of human KU812 or HMC-1 human mast cell-basophil lines results in elevated levels of secreted RANTES, MIP-1α, and MIP-1β, but not IL-8[83]. These results may suggest a role for mast cells in the initiation of chemokine-dependent host responses to DV infection. High MIP-1α levels induced in DV infected cord blood mononuclear have been suggested to be the growth inhibitor of haematopoietic progenitor[84]. MIP-1α and MIP-1β have been induced by infection with DV in a myelomonocytic cell line, as well as in peripheral blood mononuclear cells isolated from a dengue naive donor. Expression of MIP-1 genes has been shown in patients with dengue disease[77]. Carr *et al*[85] have shown that supernatants from DV-infected Mϕ contain factors that increase human umbilical vein endothelial cell permeability, but this is not accompanied by endothelial cell infection. Moreno *et al*[86] analyzed the gene expression of different chemokines, cytokines, adhesion molecules, chemokine and cytokine receptors, as well as cytokine-related molecules in an *in vitro* DV infection of human Mϕ. Transcripts for IL-8, IL-1β, osteopontin, GRO-α, -β and -γ, I-309, and some other molecules are upregulated upon infection, whereas others such as MIC-1, CD27L and CD30L, are downregulated. This pointed out 25 Mϕ-expressed cytokine-related genes that could be relevant in DV pathogenesis[86].

Role of macrophage in DV-specific cytokines

During DV infection several cytokines are produced that are unique to dengue and have not been described in any other virus infection. These include the cytokines of the cytotoxic pathway and the suppressor pathway as discussed below. Besides producing one of these cytokines, Mϕ play an important role in transmission of cytokine signal amplifying the effects of cytokines.

Role of macrophage in cytotoxic pathway in dengue

As reviewed by Chaturvedi *et al*[87] a unique cytokine, cytotoxic factor (CF) is produced by CD4+ T cells during dengue virus infection of mice and man. The aminoterminal sequence of CF has no homology with any of the known proteins or cytokines. CF selectively kills $CD4^+$ T cells and H-$2A^-$ Mϕ and induces H-$2A^+$ Mϕ to produce another cytokine, the Mϕ cytotoxin (CF2) that amplifies the effect of CF (Fig. 1). The CF purified from the sera of DHF patients, when inoculated into mice increases capillary permeability and damages the blood-brain barrier indicating its role in pathogenicity. CF and CF2 appear to be pathogenesis-related proteins, capable of reproducing DHF-like pathological lesions in mice, such as increased capillary permeability, cerebral oedema, and blood leukocyte changes[87]. Receptors for CF2 (CF-2R) have been identified on different cells besides the Mϕ[88,89]. 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. Peripheral blood mononuclear cells of such patients cultured *ex vivo* show production of CF by CD4+ T cells[90]. The production of CF precedes the clinical illness in mice and man. The DHF-like pathological lesions produced by CF/CF2 can be

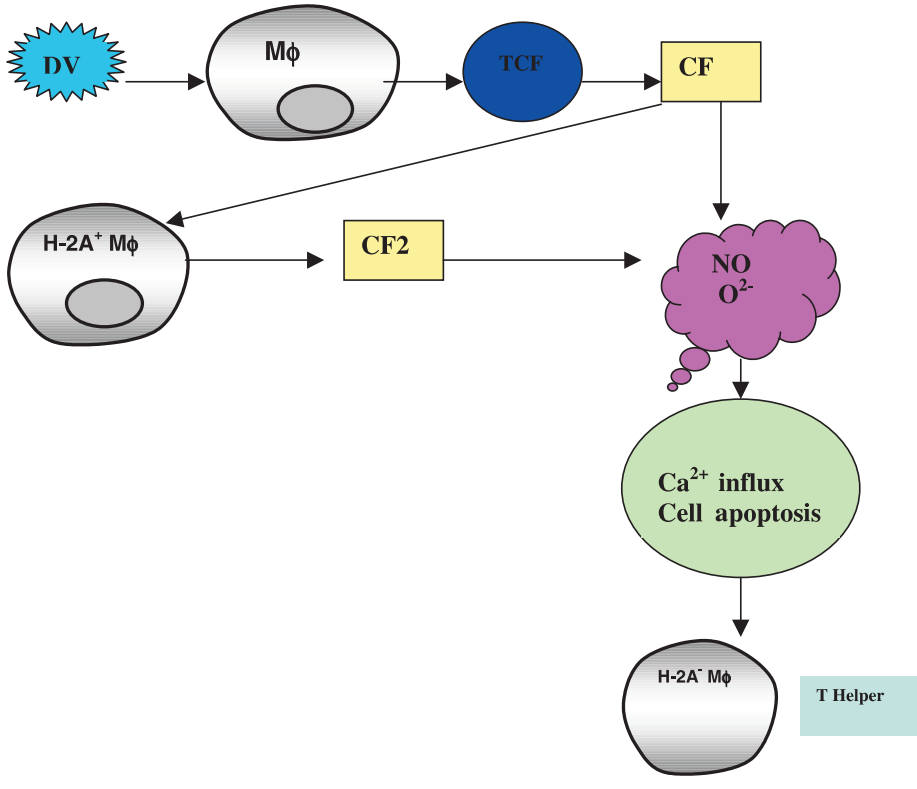

Fig. 1. Dengue virus (DV)-induced cytotoxic pathway. DV replicates in macrophages (Mϕ) and recruits CD4+ T cells (TCF) which produce the cytotoxic factor (CF). CF induces H2-A+ Mϕ to produce another cytokine the Mϕ cytotoxin (CF2). CF/CF2 induce production of free radicals that damage H2-A- Mϕ and helper T cells.

prevented by pre-treatment 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[91]. Further, highest levels of CF-autoantibodies are seen in sera of patients with mild illness (DF) while the levels decline sharply with the development of DHF and the levels are lowest in patients with DHF grade IV. This suggests that higher levels of CF-autoantibodies protect the patients against the development of DHF and may be used as a prognostic indicator[92]. Thus, DV replicates in Mϕ and induces quickly the CD4+ T cells and then Mϕ to produce a unique cytokines, CF/CF2 that induce Mϕ to produce free radicals, nitrite, reactive oxygen and peroxynitrite[93-96]. The free radicals, besides killing the target cells by apoptosis also directly upregulate production of proinflammatory cytokines IL-1β, TNF-α, IL-8, and hydrogen peroxide in Mϕ. 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 and death of patients (Fig. 2). The vascular permeability is increased due to the combined effect of histamine, free radicals, proinflammatory cytokines and the products of the complement pathway, *etc*[58]. Thus the key player appears to be CF/CF2, but their activity is regulated by CF-autoantibodies[92].

Role of macrophage in suppressor pathway in dengue

DV-infected mice develop DV antigen-specific immunosuppression, which has been shown to be mediated by a cascade of three generations of suppressor T cells (TS) and their secretary soluble suppressor cytokines (SF) with in between Mϕ transmitting the signals (Fig. 3). DV-infected Mϕ transmit the signal to recruit TS1 cells, which secrete

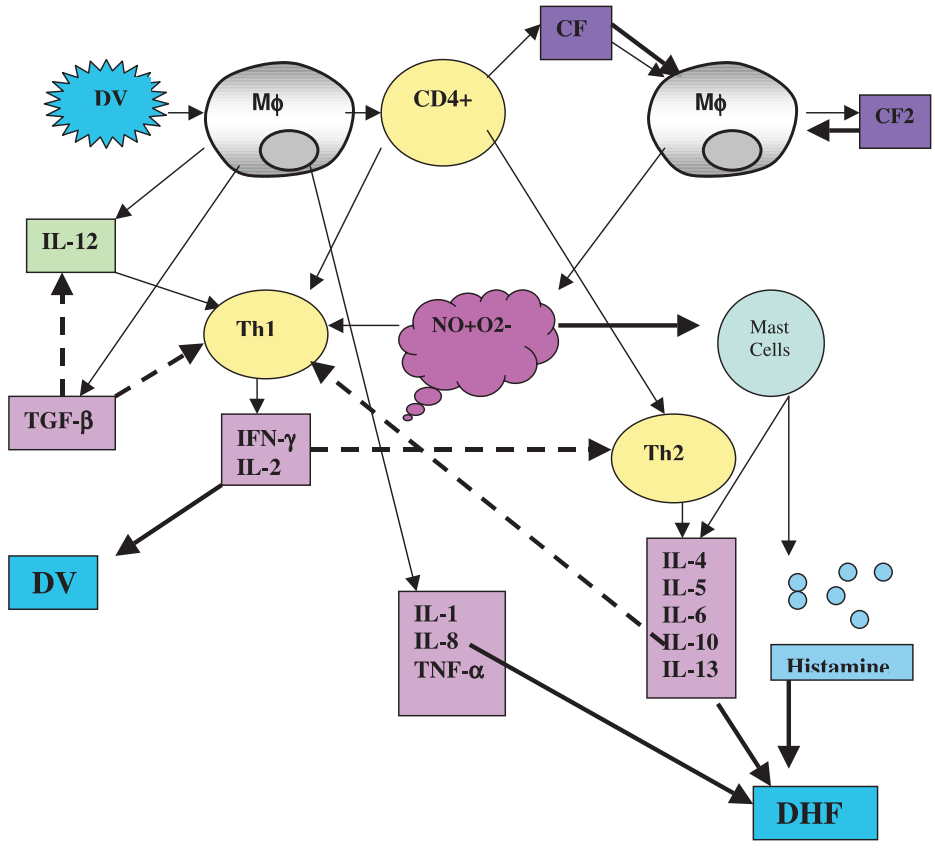

Fig. 2. Schematic presentation of the interaction of various cytokines during dengue virus infection. Any shift in the bias towards Th2- response precipitates dengue haemorrhagic fever (DHF). Thin lines, positive induction; Thick lines, damaging effect; Interrupted lines, inhibition.

a suppressor cytokine, SF1. The suppressor signal of SF is transmitted via live syngeneic Mϕ to recruit a second subpopulation of suppressor T cells (TS2), which produce another soluble, prostaglandin-like suppressor cytokine (SF2). Acting via Mϕ, the 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 via action on B cells and T helper cells[87,97-106]. Suppression of enhancing antibody by the suppressor pathway would be beneficial to body as ADE mediated DV replication is prevented. On the other hand, suppression of neutralizing antibody would delay elimination of DV from the body.

Role of macrophage in transmission of DV-specific cytokine signal

Cytokines transmit their signal via receptors on target cells. The receptors studied for DV-induced cytokines are presented in Table III. The study undertaken to investigate the intermediary role of Mϕ in transmission of signal from TS1 to TS2 showed that live syngeneic macrophage adsorb SF and transmit the signal to naïve T cells to recruit TS2. It is not possible with killed Mϕ or in absence of live Mϕ[98]. Further, transmission of suppressor signal from SF-adsorbed Mϕ to lymphocytes occurs only by physical contact of the plasma membranes of the interacting cells and not if they are separated by cell-impermeable membranes[99]. Pre-treatment of Mϕ with the calcium channel blockers that block the influx of calcium inhibits transmission of the suppressor signal from TS1 to TS2 cells in a dose-dependent manner. Presence of calcium ion is obligatory for the transmission of the suppressor signal[107]. Similarly, nitric oxide (NO) also serves as an intracellular signal in transmission of suppressor signal in Mϕ[106].

SF is composed of two polypeptide chains (α and β)[108]. Scatchard analysis showed the presence of both high and low affinity SF receptor sites (SF-R) on Mϕ. SF-R purified from normal mouse peritoneal Mϕ is

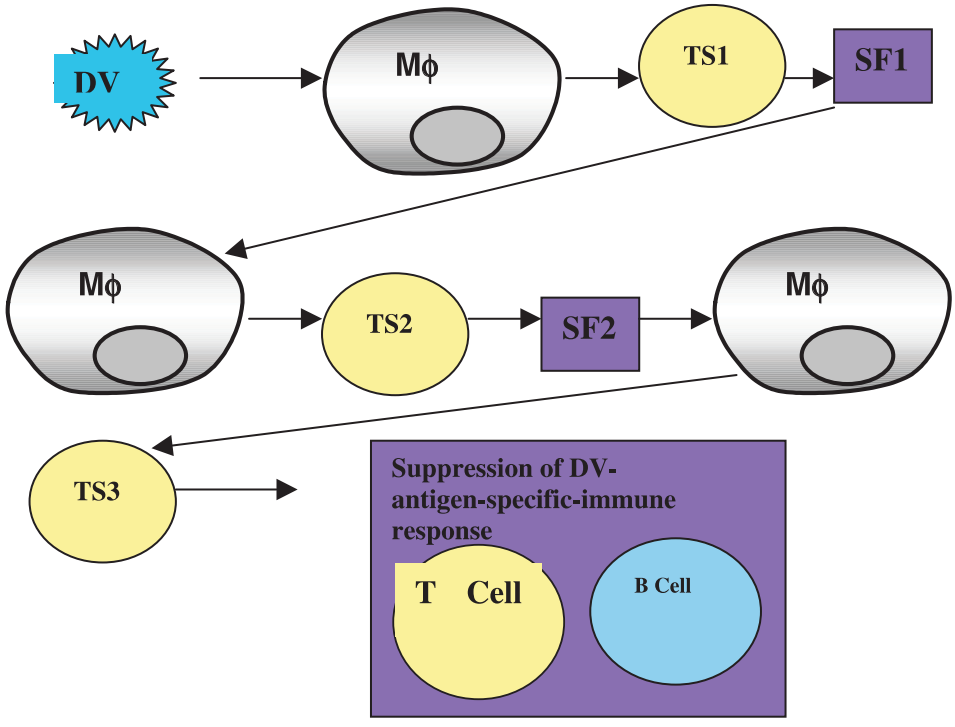

Fig. 3. DV-induced suppressor pathway. A sequential generation of three subpopulations of suppressor T cells (TS) occurs with production of soluble suppressor factor (SF) by each TS subpopulation. The signal to recruit the next subpopulation of TS is transmitted by live syngeneic Mϕ. It leads to suppression of DV-specific antibody production and the helper T cells (Th).

composed of two polypeptide chains (α and β), which are obtained in pure form by high performance liquid chromatography (HPLC) of dithiothreitol- and iodoacetamide-treated SF-R[102]. Both high and low affinity receptors are present on T and B cells[104]. Both, the α and β-chains of SF purified by HPLC, bind to Mϕ, but only α-chain binds to SF-R protein while the β-chain of SF binds to H-2A determinants on Mϕ[103]. SF binds to both high and low affinity SF-R on Mϕ and that bound to high affinity receptors are internalized through receptor-mediated endocytosis. Pre-treatment of Mϕ with anti-SF-R-antiserum and didansylcadaverine, a potent inhibitor of endocytosis inhibits this. Internalized SF is degraded by lysosomal activity and is transported to a site other than SF-R on Mϕ membrane for recruitment of TS2 cells. As SF requires binding to H-2A and SF-R for mediation of suppression, the binding of H-2A occurs with degraded SF within the cell. Thus, SF is internalized, degraded and binds to H-2K antigen before its recognition by native T cells[105]. The helper T cells (Th) generated in DV infection of mice produce a soluble helper cytokine (HF), which enhances the clonal expansion of DV-specific IgM antibody plaque forming cells (PFC). A study undertaken to investigate the mechanism of transmission of the helper signal from Th and HF to B cells showed that Th can transmit the helper signal to B cells by direct cell to cell contact, but HF cannot do so in absence of live Mϕ. HF has two polypeptide chains and both of these bind to Mϕ. HF remains on the surface of Mϕ and can be retrieved completely by contact with B cells for 40 min[109,110].

Role of macrophage in production of free radicals in DV infection

CF/CF2 induce Mϕ of mice to produce superoxide anion (O2-), hydrogen peroxide (H_2O_2) and nitrite (NO) *in vitro* and *in vivo*. It has been shown that the cytotoxic activity of CF/CF2 is mediated via these free radicals, possibly by generation of peroxynitrite[93-96]. NO and Ca^{2+} also serve as intracellular signal in transmission of DV-induced suppressor signal by Mϕ to T cells[107] (Fig. 2). Rodriguez[111] has discussed evidences that link NO with the pathology of the severe dengue disease. Ray *et al*[112] have reported alterations in the antioxidant status in the patients with acute dengue illness. DV is capable of inducing increased levels of NO when co-cultured with human KC and spleen cells. Increased levels of NO are found in patients with DF and not in patients with DHF. *In vitro* studies show no increase in levels of NO when human platelets are incubated with DV. In a patient with DF an increase in levels of NO may be an indicator of the evolution from the nonhaemorrhagic to the

Table III. Receptors on macrophage for DV-specific cytokines

Cytokine	Number of receptors/ cell (affinity)	Number of chains in cytokine	Number of chains in receptor	Sites of binding of cytokine on cells	References
Supressor factor (SF)	M: 54,000 (HA) M:1.78x10^6(LA) T: 35,000 (HA) T: 0.72x10^6(LA) B: 16,000 (HA) B:0.33x10^6(LA)	α chain β chain	α chain β chain	SFα: SFR bSFβ: H-2A	102-105
Helper factor (HF)	Present on M and B,Number not known	α chain β chain	α chain β chain	HFα+AG HFβ+H-2A	109,110, 117-119
Macrophage cytotoxin (CF2)	M: 1.1x 10^6 (IF) T: 22,000 (HA)	Not known	Not known	Binds ot M and T	88,89

AG, DV antigen; M, macrophage; T, T lymphocyte; B, B lymphocyte; HA, high affinity receptor; LA, low affinity receptor; IF, intermediate affinity receptor; SFR, receptor for SF

haemorrhagic forms of dengue[113]. NO is well known for inhibiting viral dissemination. Charnsilpa *et al*[47] have reported that NO from exogenous NO donor downregulates replication of DV and it is at the level of viral RNA and protein synthesis. This indicates that NO may serve as a defense, which diminishes viral load in patients. In DV infection endothelial cells undergo apoptosis via the mitochondria-dependent pathway that is regulated by NO production[114]. The results of Jan *et al*[115] indicate that DV infection of human neuroblastoma cells triggers an apoptotic pathway through phospholipase A (2) activation to superoxide anion generation and subsequently to NF-kappaB activation. This apoptotic effect can be either directly derived from the action of arachidonic acid and superoxide anion on mitochondria or indirectly derived from the products of apoptosis-related genes activated by NF-kappaB.

Differences in host susceptibility to infectious disease and disease severity cannot be attributed solely to the virulence of the virus. Variations in immune response, often associated with polymorphism in the human genome can be detected. The data concerning the influence of human genes in DF and DHF have been discussed in a recent review[116] showing the associations between HLA polymorphism and dengue disease susceptibility or resistance; protective alleles influencing progression to severe disease; alleles restricting CD4+ and CD8+ T lymphocytes and non-HLA genetic factors that may contribute to DHF evolution, *e.g.*, genes influencing various cytokines production and Mϕ functions during DV infection[116]. Table IV summarizes the role of macrophages in DV infection.

Conclusions

The ideal situation would be that the invading virus is ingested, digested and eliminated by Mϕ. In the case of DV, in addition to these functions spanning the spectrum of innate and adaptive immune responses, Mϕ also serve as the host cells for efficient replication of DV, complicating the immune functions. A successful challenge to virus infection requires that a balance is achieved between the induction of efficient anti-viral effector mechanisms and the avoidance of detrimental tissue damage. The sensor of infectious invasion in innate immune system is Toll-like receptor (TLR), while Mϕ get signals via the Jak-Stat pathway [Janus kinase (Jak)-signal transducer and activation of transcription

Table IV. Beneficial and harmful effects of macrophages during DV infection

Functions of Mf	Beneficial effects	Harmful effects
Innate immune response	Phagocytosis, ingestion, digestion and elimination of DV; Activation of Mϕ; Killing of DV-infected cells; Secretion of cytokines that inhibit virus replication	Replication of DV; killing of healthy cells in the neighbourhood of infection; Depression of Mϕ functions
Adaptive immune response	DV antigen presentation to B and T lymphocytes to generate specific immune response; cytotoxic T lymphocytes kill DV-infected cells	Replication of DV; Cytotoxic T lymphocytes may cause extensive tissue damage
Cytotoxic pathway	Production of anti-CF-antibody neutralizes CF	Killing of Mϕ and Th cells; Increased capillary permeability leading to haemorrhage, oedema and shock
Suppressor pathway	Suppression of enhancing antibody (no ADE)	Suppression of neutralizing antibody
Cytokines	Generation of Th1-type response; IL-12; IFN-γ	Generation of Th2-type response; IL-6, IL-8, TGFβ1; suppression of IFN-α
Free radicals	May restrict virus replication	Apoptosis of cells; Increased capillary permeability

ADE, antibody dependent enhancement of DV replication; CF, cytotoxic factor; IL, interleukin; Mϕ, macrophage; Th, helper T cells

(Stat) pathway] for activation or inhibition. Key to the control of viral infections is IFN-γ which depends on functional Stat1 signal transduction. Stat3 signaling is activated by a range of cytokines, including IL-10, IL-6 and IL-27. Recent progress in understanding the regulation of Mϕ function in infection by Stat-activating cytokines, their receptors or signaling components indicate the importance of the Stat-pathway in the control of infection and immunopathology. With the advances in genomics and availability of newer technology it may be possible to define the molecular mechanisms of Mϕ activation and depression. It may now be possible in near future to characterize the target genes and identify the molecular mediators that may inhibit subversion of Mϕ by viruses (and intracellular pathogens) and control tissue destruction.

Acknowledgment

Most of the work reported was supported by the Indian Council of Medical Research (ICMR), Council of Scientific & Industrial Research (CSIR), Department of Science & Technology & Department of Biotechnology, Government of India, New Delhi to one of us (UCC).

References

1. Agarwal R, Kapoor S, Nagar R, Misra A, Tandon R, Mathur A, *et al*. A clinical study of the patients with dengue haemorrhgic fever during the epidemic of 1996 at Lucknow, India. *Southeast Asian J Trop Med Public Health* 1999; *30* : 735-40.

2. Rigau-Perez JG, Clark GG, Gubler DJ, Reiter P, Sanders EJ, Vorndam AV. Dengue and dengue haemorrhagic fever. *Lancet* 1998; *352* : 971-7.

3. Malavige GN, Fernando S, Fernando DJ, Seneviratne SL. Dengue viral infections. *Postgrad Med J* 2004; *80* : 588-601.

4. Halstead SB. Pathophysiology and pathogenesis of dengue haemorrhagic fever. In: Thongcharoen P, editor. *Monograph on dengue/dengue haemorrhagic fever*. New Delhi: WHO-SEARO; 1993 p. 80-103.

5. Halstead SB. Dengue. *Curr Opin Infect Dis* 2002; *15* : 471-6.

6. Chaturvedi UC, Shrivastava R. Dengue haemorrhagic fever: A global challenge. *Indian J Med Microbiol* 2004; *22* : 5-6.

7. Chaturvedi UC, Rizvi N, Mathur A. Antigen presentation. *Curr Sci* 1987; *56* : 561-8.

8. Chaturvedi UC, Nagar R, Mathur A. Effect of dengue virus infection on Fc-receptor functions of mouse macrophages. *J Gen Virol* 1983; *64* : 2399-407.

9. Kwan WH, Helt AM, Maranon C, Barbaroux JB, Hosmalin A, Harris E, *et al*. Dendritic cell precursors are permissive to dengue virus and human immunodeficiency virus infection. *J Virol* 2005; *79* : 7291-9.

10. Marovich M, Grouard-Vogel G, Louder M, Eller M, Sun W, Wu SJ, *et al*. Human dendritic cells as targets of dengue virus infection. *J Invest Dermatol Symp Proc* 2001; *6* : 219-24.

11. Schaerli P, Willimann K, Ebert LM, Walz A, Moser B. Cutaneous CXCL14 targets blood precursors to epidermal niches for langerhans cell differentiation. *Immunity* 2005; *23* : 331-42.

12. Wu SJ, Grouard-Vogel G, Sun W, Mascola JR, Brachtel E, Putvatana R, *et al*. Human skin Langerhans cells are targets of dengue virus infection. *Nat Med* 2000; *6* : 816-20.

13. Ramos C, Sanchez G, Pando RH, Baquera J, Hernandez D, Mota J, *et al*. Dengue virus in the brain of a fatal case of hemorrhagic dengue fever. *J Neurovirol* 1998; *4* : 465-8.

14. Combarros O, Infante J, Rodriguez E, Llorca J, Pena N, Fernandez-Viadero C, *et al*. CD14 receptor polymorphism and Alzheimer's disease risk. *Neurosci Lett* 2005; *380* : 193-6.

15. Marianneau P, Steffan AM, Royer C, Drouet MT, Jaeck D, Kirn A, *et al*. Infection of primary cultures of human Kupffer cells by dengue virus: no viral progeny synthesis, but cytokine production is evident. *J Virol* 1999; *73* : 5201-6.

16. Koning GA, Morselt HW, Gorter A, Allen TM, Zalipsky S, Scherphof GL, *et al*. Interaction of differently designed immunoliposomes with colon cancer cells and Kupffer cells. An *in vitro* comparison. *Pharm Res* 2003; *20* : 1249-57.

17. Mogensen SC. Role of macrophages in natural resistance to virus infection. *Microbiol Rev* 1979; *43* : 1-26.

18. Fujiwara N, Kobayashi K. Macrophages in inflammation. *Curr Drug Targ - Inflam Aller* 2005; *4* : 281-6.

19. Gulati L, Chaturvedi UC, Mathur A. Depressed macrophage functions in dengue virus-infected mice: role of the cytotoxic factor. *Br J Exp Pathol* 1982; *63* : 194-202.

20. Nagar R, Chaturvedi UC, Mathur A. Effect of dengue virus-induced cytotoxic factor on Fc-receptor functions of mouse macrophages. *Br J Exp Pathol* 1984; *65* : 11-7.

21. Theofilopoulos AN, Brandt WE, Russell PK, Dixon FT. Replication of dengue-2 virus in cultured human lymphoblastoid cells and subpopulations of human peripheral leukocytes. *J Immunol* 1976; *117* : 953-61.

22. Daughaday C, Brandt WE, McCown JM, Russell PK. Evidence for two mechanisms of dengue virus infection of adherent human monocytes: trypsin-sensitive virus receptors and trypsin-resistant immune complex receptors. *Infect Immun* 1981; *32* : 469-73.

23. Schlesinger JJ, Chapman SE. Influence of the human high-affinity IgG receptor FcgammaRI (CD64) on residual infectivity of neutralized dengue virus. *Virology* 1999; *260* : 84-8.

24. Moreno-Altamirano MM, Sanchez-Garcia FJ, Munoz ML. Non Fc receptor-mediated infection of human macrophages by dengue virus serotype 2. *J Gen Virol* 2002; *83* : 1123-30.

25. Reyes-Del Valle J, Chavez-Salinas S, Medina F, Del Angel RM. Heat shock protein 90 and heat shock protein 70 are components of dengue virus receptor complex in human cells. *J Virol* 2005; *79* : 4557-67.

26. Nakao S, Lai CJ, Young NS. Dengue virus, a flavivirus, propagates in human bone marrow progenitors and hematopoietic cell lines. *Blood* 1989; *74* : 1235-40.

27. Pryor MJ, Carr JM, Hocking H, Davidson AD, Li P, Wright PJ. Replication of dengue virus type 2 in human monocyte-derived macrophages: comparisons of isolates and recombinant viruses with substitutions at amino acid 390 in the envelope glycoprotein. *Am J Trop Med Hyg* 2001; *65* : 427-34.

28. Cologna R, Rico-Hesse R. American genotype structures decrease dengue virus output from human monocytes and dendritic cells. *J Virol* 2003; *77* : 3929-38.

29. Mosquera JA, Hernandez JP, Valero N, Espina LM, Anez GJ. Ultrastructural studies on dengue virus type 2 infection of cultured human monocytes. *Virol J* 2005; *2* : 26-32.

30. Ho LJ, Hung LF, Weng CY, Wu WL, Chou P, Lin YL, *et al*. Dengue virus type 2 antagonizes IFN-alpha but not IFN-gamma antiviral effect via down-regulating Tyk2-STAT signaling in the human dendritic cell. *J Immunol* 2005; *174* : 8163-72.

31. Shresta S, Sharar KL, Prigozhin DM, Snider HM, Beatty PR, Harris E. Critical roles for both STAT1-dependent and STAT1-independent pathways in the control of primary dengue virus infection in mice. *J Immunol* 2005; *175* : 3946-54.

32. Halstead SB. Observations related to pathogenesis of dengue hemorrhagic fever. VI. Hypotheses and discussion. *Yale J Biol Med* 1970; *42* : 350-62.

33. Halstead SB, Nimmannitya S, Cohen SN. Observations related to pathogenesis of dengue hemorrhagic fever. IV. Relation of disease severity to antibody response and virus recovered. *Yale J Biol Med* 1970; *42* : 311-28.

34. Halstead SB, O'Rourke EJ. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. *J Exp Med* 1977; *146* : 201-17.

35. Sangkawibha N, Rojanasuphot S, Ahandrik S, Viriyapongse S, Jatanasen S, Salitul V, *et al*. Risk factors for dengue shock syndrome: a prospective epidemiologic study in Rayong, Thailand. I. The 1980 outbreak. *Am J Epidemiol* 1984; *120* : 653-69.

36. Guzman MG, Kouri GP, Bravo J, Soler M, Vazquez S, Morier L. Dengue hemorrhagic fever in Cuba, 1981: a retrospective seroepidemiologic study. *Am J Trop Med Hyg* 1990; *42* : 179-84.

37. Burke DS, Nisalak A, Johnson DE, Scott RM. A prospective study of dengue infections in Bangkok. *Am J Trop Med Hyg* 1988; *38* : 172-80.

38. Halstead SB. *In vivo* enhancement of dengue virus infection in rhesus monkeys by passively transferred antibody. *J Infect Dis* 1979; *140* : 527-33.

39. Morens DM, Halstead SB. Disease severity-related antigenic differences in dengue 2 strains detected by dengue 4 monoclonal antibodies. *J Med Virol* 1987; *22* : 169-74.

40. Murgue B, Roche C, Chungue E, Deparis X. Prospective study of the duration and magnitude of viraemia in children hospitalised during the 1996-1997 dengue-2 outbreak in French Polynesia. *J Med Virol* 2000; *60* : 432-8.

41. Libraty DH, Endy TP, Houng HS, Green S, Kalayanarooj S, Suntayakorn S, *et al*. Differing influences of viral burden and immune activation on disease severity in secondary dengue 3 virus infections. *J Infect Dis* 2002; *185* : 1213-21.

42. Peiris JS, Porterfield JS. Antibody-mediated enhancement of Flavivirus replication in macrophage-like cell lines. *Nature* 1979; *282* : 509-11.

43. Hotta H, Hotta S. Dengue virus multiplication in cultures of mouse peritoneal macrophages: effects of macrophage activators. *Microbiol Immunol* 1982; *26* : 665-76.

44. Hotta H, Homma M. Lectin-mediated enhancement of dengue virus infection in a mouse macrophage cell line Mk1. *Arch Virol* 1994; *134* : 51-9.

45. Hotta H, Sanchez LF, Takada H, Homma M, Kotani S. Enhancement of dengue virus infection in cultured mouse macrophages by lipophilic derivatives of muramyl peptides. *Microbiol Immunol* 1985; *29* : 533-41.

46. Chen YC, Wang SY, King CC. Bacterial lipopolysaccharide inhibits dengue virus infection of primary human monocytes/ macrophages by blockade of virus entry via a CD14-dependent mechanism. *J Virol* 1999; *73* : 2650-7.

47. Charnsilpa W, Takhampunya R, Endy TP, Mammen MP Jr, Libraty DH, Ubol S. Nitric oxide radical suppresses replication of wild-type dengue 2 viruses *in vitro. J Med Virol* 2005; *77* : 89-95.

48. Rizvi N, Chaturvedi UC, Nagar R, Mathur A. Macrophage functions during dengue virus infection: antigenic stimulation of B cells. *Immunology* 1987; *62* : 493-8.

49. Rizvi N, Chaturvedi UC, Mathur A. Obligatory role of macrophages in dengue virus antigen presentation to B lymphocytes. *Immunology* 1989; *67* : 38-43.

50. Rizvi N, Chaturvedi UC, Mathur A. Inhibition of the presentation of dengue virus antigen by macrophages to B cells by serine-protease inhibitors. *Int J Exp Pathol* 1991; *72* : 23-9.

51. Rizvi N, Chaturvedi UC, Mathur A. Antigenic competition between dengue and Coxsackie viruses for presentation to B cells by macrophages. *Int J Exp Pathol* 1990; *71* : 761-70.

52. Taweechaisupapong S, Sriurairatana S, Angsubhakorn S, Yoksan S, Khin MM, Sahaphong S, *et al*. Langerhans cell density and serological changes following intradermal immunisation of mice with dengue 2 virus. *J Med Microbiol* 1996; *45* : 138-45.

53. Taweechaisupapong S, Sriurairatana S, Angsubhakorn S, Yoksan S, Bhamarapravati N. *In vivo* and *in vitro* studies on the morphological change in the monkey epidermal Langerhans cells following exposure to dengue 2 (16681) virus. *Southeast Asian J Trop Med Public Health* 1996; *27* : 664-72.

54. Palmer DR, Sun P, Celluzzi C, Bisbing J, Pang S, Sun W, *et al*. Differential effects of dengue virus on infected and bystander dendritic cells. *J Virol* 2005; *79* : 2432-9.

55. Lozach PY, Burleigh L, Staropoli I, Navarro-Sanchez E, Harriague J, Virelizier JL, *et al*. Dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN)-mediated enhancement of dengue virus infection is independent of DC-SIGN internalization signals. *J Biol Chem* 2005; *280* : 23698-708.

56. Chaturvedi UC, Raghupathy R, Pacsa AS, Elbishbishi EA, Agarwal R, Nagar R, *et al*. Shift from a Th1-type response to Th2-type in dengue haemorrhagic fever. *Curr Sci* 1999; *76* : 63-9.

57. Chaturvedi UC, Elbishbishi EA, Agarwal R, Raghupathy R, Nagar R, Tandon R, *et al*. Sequential production of cytokines by dengue virus-infected human peripheral blood leukocyte cultures. *J Med Virol* 1999; *59* : 335-40.

58. Chaturvedi UC, Agarwal R, Elbishbishi EA, Mustafa AS. Cytokine cascade in dengue haemorrhagic fever: Implications for pathogenesis. *FEMS Immunol Med Microbiol* 2000; *28* : 183-8.

59. Chaturvedi UC, Shrivastava R, Nagar R. Dengue vaccines: Prospects and problems. *Indian J Med Res* 2005; *121* : 639-52.

60. Hober D, Poli L, Roblin B, Gestas P, Chungue E, Granic G, *et al*. Serum levels of tumor necrosis factor-α (TNF-α), Interleukin-6 (IL-6), and Interleukin-1β (IL-1 β) in dengue infected patients. *Am J Trop Med Hyg* 1993; *48* : 324-31.

61. Kuno G, Bailey RE. Cytokine rsponses to dengue infection among Puert Rican patients. *Mem Inst Oswaldo Cruz* 1994; *89* : 179-82.

62. Ingkaran N, Yadav M, Sinniah M. Augmented inflammatory cytokines in primary dengue infection progressing to shock. *Singapore Med J* 1995; *36* : 218-21.

63. Laur F, Murgue B, Deparis X, Roche C, Cassar O, Chungue E. Plasma levels of tumour necrosis factor alpha and trnsforming growth factor beta-1 in children with dengue 2 virus infection in French Polynesia. *Trans R Soc Trop Med Hyg* 1998; *92* : 654-6.

64. Bethell DB, Flobbe K, Cao XT, Day NP, Pham TP, Buurman WA, *et al*. Pathophysiologic and prognostic role of cytokines in dengue hemorrhagic fever. *J Infect Dis* 1998; *177* : 778-82.

65. Nguyen TH, Lei HY, Nguyen TL, Lin YS, Huang KJ, Le BL, *et al*. Dengue hemorrhagic fever in infants: a study of clinical and cytokine profiles. *J Infect Dis* 2004; *189* : 221-32.

66. Lin CF, Chiu SC, Hsiao YL, Wan SW, Lei HY, Shiau AL, *et al*. Expression of cytokine, chemokine, and adhesion molecules during endothelial cell activation induced by antibodies against dengue virus nonstructural protein 1. *J Immunol* 2005; *174* : 395-403.

67. Raghupathy R, Chaturvedi UC, Al-Sayer H, Elbishbishi EA, Agarwal R, Nagar R, *et al*. Elevated levels of IL-8 in dengue hemorrhagic fever. *J Med Virol* 1998; *56* : 280-5.

68. Green S, Vaughn DW, Kalayanarooj S, Nimmannitya S, Suntayakorn S, Nisalak A, *et al*. Elevated plasma interleukin levels in acute dengue correlate with disease severity. *J Med Virol* 1999; *59* : 329-34.

69. Pacsa AS, Agarwal R, Elbishbishi EA, Chaturvedi UC, Nagar R, Mustafa AS. Interleukin-12 in patients with dengue haemorrhagic fever. *FEMS Immunol Med Microbiol* 2000; *28* : 151-5.

70. Mustafa AS, Elbishbishi EA, Agarwal R, Chaturvedi UC. Elevated levels of interleukin-13 and IL-18 in patients with dengue hemorrhagic fever. *FEMS Immunol Med Microbiol* 2001; *30* : 229-33.

71. Kittigul L, Temprom W, Sujirarat D, Kittigul C. Determination of tumor necrosis factor-alpha levels in dengue virus infected patients by sensitive biotin-streptavidin enzyme-linked immunosorbent assay. *J Virol Methods* 2000; *90* : 51-7.

72. Pinto LM, Oliveira SA, Braga EL, Nogueira RM, Kubelka CF. Increased proinflammatory cytokines (TNF-alpha and IL-6) and anti-inflammatory compounds (sTNFRp55 and sTNFRp75) in Brazilian patients during exanthematic dengue fever. *Mem Inst Oswaldo Cruz* 1999; *94* : 387-94.

73. Agarwal R, Elbishbishi EA, Chaturvedi UC, Nagar R, Mustafa AS. Profile of transforming growth factor-beta1 in patients with dengue haemorrhagic fever. *Int J Exp Pathol* 1999; *80* : 143-9.

74. Gulati L, Chaturvedi UC, Mathur A. Dengue virus-induced cytotoxic factor induces macrophages to produce a cytotoxin. *Immunology* 1983; *49* : 121-30.

75. Gulati L, Chaturvedi UC, Mathur A. Production of dengue virus-induced macrophage cytotoxin *in vivo. Br J Exp Pathol* 1986; *67* : 269-77.

76. Dhawan R, Khanna M, Chaturvedi UC, Mathur A. Effect of dengue virus-induced cytotoxin on capillary permeability. *Br J Exp Pathol (Oxford)* 1990; *71* : 83-8.

77. Spain-Santana TA, Marglin S, Ennis FA, Rothman AL. MIP-1 alpha and MIP-1 beta induction by dengue virus. *J Med Virol* 2001; *65* : 324-30.

78. Komastu T, Ireland DDC, Reiss CS. IL-12 and viral infections. *Cytokines Growth Factor Rev* 1998; *9* : 277-85.

79. Juffrie M, van Der Meer GM, Hack CE, Haasnoot K, Sutaryo, Veerman AJ, *et al*. Inflammatory mediators in dengue virus infection in children: interleukin-8 and its relationship to neutrophil degranulation. *Infect Immun* 2000; *68* : 702-7.

80. Huang YH, Lei HY, Liu HS, Lin YS, Liu CC, Yeh TM. Dengue virus infects human endothelial cells and induces IL-6 and IL-8 production. *Am J Trop Med Hyg* 2000; *63* : 71-5.

81. Medin CL, Fitzgerald KA, Rothman AL. Dengue virus nonstructural protein NS5 induces interleukin-8 transcription and secretion. *J Virol* 2005; *79* : 11053-61.

82. Chen YC, Wang SY. Activation of terminally differentiated human monocytes/macrophages by dengue virus: productive infection, hierarchical production of innate cytokines and chemokines, and the synergistic effect of lipopolysaccharide. *J Virol* 2002; *76* : 9877-87.

83. King CA, Anderson R, Marshall JS. Dengue virus selectively induces human mast cell chemokine production. *J Virol* 2002; *76* : 8408-19.

84. Murgue B, Cassar O, Deparis X, Guigon M, Chungue E. Implication of macrophage inflammatory protein-1alpha in the inhibition of human haematopoietic progenitor growth by dengue virus. *J Gen Virol* 1998; *79* : 1889-93.

85. Carr JM, Hocking H, Bunting K, Wright PJ, Davidson A, Gamble J, *et al*. Supernatants from dengue virus type-2 infected macrophages induce permeability changes in endothelial cell monolayers. *J Med Virol* 2003; *69* : 521-8.

86. Moreno-Altamirano MM, Romano M, Legorreta-Herrera M, Sanchez-Garcia FJ, Colston MJ. Gene expression in human macrophages infected with dengue virus serotype-2. *Scand J Immunol* 2004; *60* : 631-8.

87. Chaturvedi UC, Dhawan R, Mukerjee R. Immunosuppression and cytotoxicity of dengue infection in the mouse model. In: Gubler DJ, Kuno G, editors. *Dengue and dengue haemorrhagic fever*. Wallingford, Oxon, UK: CAB International Press; 1997 p. 291-312.

88. Khare PD, Dhawan R, Chaturvedi UC. Identification and characterization of receptor for dengue virus-indiced macrophage cytotoxin. *Indian J Exp Biol* 1996; *34* : 651-7.

89. Khare PD, Khare M, Tandon R, Chaturvedi UC. Identification, purification and characterization of a receptor for dengue virus-induced macrophage cytotoxin (CF2) from murine T cells. *FEMS Immunol Med Microbiol* 2003; *38* : 35-43.

90. Agarwal R, Chaturvedi UC, Misra A, Mukerjee R, Kapoor S, Nagar R, *et al*. Production of cytotoxic factor by peripheral blood mononuclear cells (PBMC) in patients with dengue haemorrhagic fever. *Clin Exp Immunol* 1998; *112* : 340-4.

91. Chaturvedi UC, Mukerjee R, Dhawan R. Active immunization by a dengue virus-induced cytokine. *Clin Exp Immunol* 1994; *96* : 202-7.

92. Chaturvedi UC, Elbishbishi EA, Agarwal R, Mustafa AS. Cytotoxic factor-autoantibodies: possible role in the pathogenesis of dengue haemorrhagic fever. *FEMS Immunol Med Microbiol* 2001; *30* : 181-6.

93. Misra A, Mukerjee R, Chaturvedi UC. Release of reactive oxygen intermediates by dengue virus-induced macrophage cytotoxin. *Int J Exp Pathol* 1996; *77* : 237-42.

94. Misra A, Mukerjee R, Chaturvedi UC. Production of nitrite by dengue virus-induced cytotoxic factor. *Clin Exp Immunol* 1996; *104* : 406-11.

95. Misra A, Mukerjee R, Chaturvedi UC. Respiratory burst by dengue virus-induced cytotoxic factor. *Med Principles Pract* 1998; *7* : 251-60.

96. Mukerjee R, Misra A, Chaturvedi UC. Dengue virus-induced cytotoxin releases nitrite by spleen cells. *Int J Exp Pathol* 1996; *77* : 45-51.

97. Shukla MI, Chaturvedi UC. Dengue virus-induced suppressor factor stimulates production of prostaglandin to mediate suppression. *J Gen Virol* 1981; *66* : 241-9.

98. Shukla MI, Chaturvedi UC. *In vivo* role of macrophages in transmission of dengue virus-induced suppressor signal to T lymphocytes. *Br J Exp Pathol* 1982; *63* : 522-30.

99. Shukla MI, Chaturvedi UC. Transmission of dengue virus-induced suppressor signal from macrophage to lymphocyte occurs by cell contact. *Br J Exp Pathol* 1983; *64* : 87-92.

100. Shukla MI, Chaturvedi UC. Study of the target cell of the dengue virus-induced suppressor signal. *Br J Exp Pathol* 1984; *65* : 267-73.

101. Shukla MI, Chaturvedi UC. Duration of adoptively transferred dengue virus-induced suppressor activity. *Br J Exp Pathol* 1985; *66* : 337-83.

102. Mukherjee R, Chaturvedi P, Chaturvedi UC. Identification of a receptor on macrophages for the dengue virus-induced suppressor cytokine. *Clin Exp Immunol* 1993; *91* : 257-65.

103. Mukherjee R, Chaturvedi P, Chaturvedi UC. Binding of the two polypeptide chains of dengue virus-induced suppressor cytokine to its receptor isolated from macrophages. *Int J Exp Pathol* 1993; *74* : 259-66.

104. Mukherjee R, Chaturvedi P, Chaturvedi UC. Specific receptor for dengue virus-induced suppressor cytokine on macrophages and lymphocytes. *Int J Exp Pathol* 1994; *75* : 29-36.

105. Tripathi RK, Khare M, Chaturvedi UC. Internalization of dengue virus- induced suppressor cytokine during transmission of the suppressor signal via macrophage. *Indian J Exp Biol* 1997; *35* : 850-4.

106. Khare M, Chaturvedi UC. Role of nitric oxide in transmission of dengue virus specific suppressor signal. *Indian J Exp Biol* 1997; *35* : 855-60.

107. Khare M, Chaturvedi UC. Transmission of dengue virus-specific suppressor signal depends on the presence of calcium. *Indian J Med Res* 1995; *102* : 1-8.

108. Bhargava A, Chaturvedi UC, Srivastava N, Mathur A. Dengue virus-induced suppressor factor has two disulphide-bonded chains which bears anti-idiotypicc and I-A and I-J determinants. *Curr Sci* 1990; *58* : 157-60.

109. Chaturvedi P, Mukherjee R, Chaturvedi UC, Mathur A. Characterization of the dengue virus-induced helper cytokine. *Int J Exp Pathol* 1992; *73* : 263-72.

110. Chaturvedi P, Chaturvedi UC, Mukherjee R. Transmission of dengue virus-induced helper signal to B cell via macrophages. *Int J Exp Pathol* 1992; *73* : 773-82.

111. Rodriguez-Ortega M. Nitric oxide in dengue pathology. *Acta Cient Venez* 1998; *49* (Suppl 1) : 8-12.

112. Ray G, Kumar V, Kapoor AK, Dutta AK, Batra S. Status of antioxidants and other biochemical abnormalities in children with dengue fever. *J Trop Pediatr* 1999; *45* : 4-7.

113. Valero N, Espina LM, Anez G, Torres E, Mosquera JA. Short report: increased level of serum nitric oxide in patients with dengue. *Am J Trop Med Hyg* 2002; *66* : 762-4.

114. Lin YS, Lin CF, Lei HY, Liu HS, Yeh TM, Chen SH, *et al*. Antibody-mediated endothelial cell damage via nitric oxide. *Curr Pharm Des* 2004; *10* : 213-21.

115. Jan JT, Chen BH, Ma SH, Liu CI, Tsai HP, Wu HC, *et al*. Potential dengue virus-triggered apoptotic pathway in human neuroblastoma cells: arachidonic acid, superoxide anion, and NF-kappaB are sequentially involved. *J Virol* 2000; *74* : 8680-91.

116. Chaturvedi UC, Nagar R, Shrivastava R. Dengue and dengue haemorrhagic fever: implications of host genetics. *FEMS Immunol Med Microbiol* 2006; *47* : 155-66.

117. Rizvi N, Chaturvedi P, Chaturvedi UC. Bindings of macrophages and B lymphocytes mediated by dengue virus antigen and the virus-induced helper cytokine. *Int J Exp Pathol* 1993; *74* : 187-94.

118. Chaturvedi UC, Dhawan R, Khanna M, Mathur A. Breakdown of the blood-brain barrier during dengue virus infection of mice. *J Gen Virol* 1991; *72* : 859-66.

119. Chaturvedi P, Mukherjee R, Chaturvedi UC, Mathur A. Dengue virus-induced helper cytokine has two polypeptide chains which bear different determinants. *Int J Exp Pathol* 1991; *72* : 665-72.

Reprint requests: Prof. U.C. Chaturvedi, 201 Annapurna Apartments, No.1, Bishop Rocky Street
Faizabad Road, Lucknow 226007, India
e-mail: ucc05@rediffmail.com; uchaturvedi@yahoo.com