MINIREVIEW

Dengue virus-specific suppressor T cells: current perspectives

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Abstract

Dengue virus was the first microorganism that was shown to induce generation of antigen-specific suppressor T (TS) cells in mice. The cascade of the three generations of TS cells (TS1, TS2, TS3) and their secretary products, the suppressor factors (SF1, SF2), was delineated. The TS pathway was proposed to be protective through inhibition of the production of enhancing antibody, which may enhance the severity of dengue disease. The currently second most favoured mechanism of severe dengue disease is the 'cytokine tsunami'. During the last decade, suppressor/regulatory T cells have been studied in greater detail using modern techniques in various diseases, including viral infections. This brief review discusses the role of dengue-specific suppressor T cells in protection and/or induction of severe dengue disease in view of our current understanding of suppressor/regulatory T cells.

Introduction

The immune response involves a complex series of interactions between macrophage-like cells (Mϕ), T and B cells, initiated by an antigenic stimulus. The modulators of the immune response are suppressor/regulatory T cells, which may be antigen-specific or nonspecific in effecter functions (Chaturvedi, 1984). The immune response is of great importance in recovery from infections but may also result in immunopathology in several infections, and therefore regulation of the immune response is crucial in infectious diseases. The earliest studies on suppressor T cells and their products mediating immunosuppression were done in the 1970s using synthetic antigens (Fig. 1). With the available technology at that time, a sequential generation of up to three subpopulations of suppressor T cells (TS1, TS2, TS3) and their secretary products (suppressor factor) were described that transmitted the signal to suppress antigen-specific humoral and/or cell-mediated immune responses (reviewed by Benacerraf & Germain, 1981). For the first time microorganism-induced suppressor T-cell cascades (Fig. 1) were delineated in dengue virus (DV)-infected mice (Tandon *et al.*, 1979; Shukla & Chaturvedi, 1981, 1984; Chaturvedi, 1984) and then in Japanese encephalitis virus (JEV)-infected mice (Mathur *et al.*, 1983, 1984). The role of suppressor T cells in preventing immunopathology in DV and their role in the persistence and latency of JEV infection were reported (Chaturvedi, 1984; Mathur *et al.*, 1987a, b). In some cases, regulatory immune responses downregulate pathogenic responses, whereas in others they enhance pathogen survival leading to chronic infection. A comparison of the suppressor T-cell cascades induced by different antigens is summarized in Fig. 1. Further understanding of suppressor T cells was restricted due to the lack of technology to detect definitive cell markers. By the late 1980s, interest in suppressor T cells had declined and their existence was even considered doubtful. An explicit narration of the rise and downfall of suppressor T cells and their re-emergence as a regulatory T cell in recent times can be found in the excellent review by Steinman (2007).

Sakaguchi *et al.* (1995) were the first to describe a naturally occurring subset of CD4+ cells that expressed interleukin-2 (IL-2) receptor α-chains (CD25) in mice. Studies demonstrated that this subset of cells inhibits antitumour immunity, prevents a wide range of autoimmune disorders including inflammatory bowel disease and

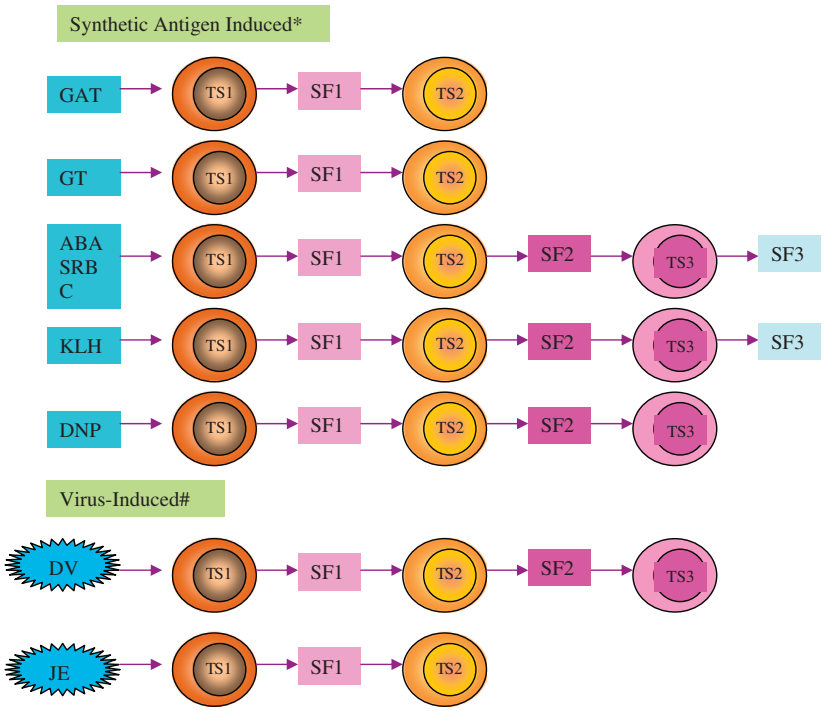

Fig. 1. Comparison of different suppressor T-cell cascades. Antigens induce a suppressor pathway in mice that involves sequential production of up to three generations of T suppressor cells (TS1, TS2, TS3). Ts1 cells produce a soluble suppressor factor (SF1), which transmits the signal via macrophages to generate TS2 cells, which produce a soluble suppressor factor (SF2) to recruit TS3 cells, which suppress antigen-specific immune response. ABA, azobenzenarsonate; DNP, dinitrophenyl; DV, dengue type 2 virus; GAT, L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10}; GT, L-glutamic acid^{50}-L-tyrosine^{50}; JE, Japanese encephalitis virus; KLH, keyhole limpet haemocyanin; SRBC, sheep red blood cells (*Benacerraf & Germain, 1981; #Chaturvedi, 1984; Chaturvedi, 1997; Mathur *et al.* 1983, 1984, 1986a).

autoimmune diabetes, and induces tolerance to skin allografts (Asano *et al.*, 1996). This vindicated the credibility of the idea of suppressor T cells and refocused attention on this subset of cells. This led to extensive characterization of regulatory T-cell properties *in vitro* and development of a simple *in vitro* assay for their functions. This made analysis of regulatory T cells possible in rodents and humans (Takahashi *et al.*, 1998; Thornton & Shevach, 1998). This subgroup of cells has since been described in humans and, as in mice, constitutes about 5–10% of peripheral CD4+ T cells (Jonuleit *et al.*, 2001). These cells can be obtained from peripheral blood, thymus and secondary lymphoid organs including tonsils and spleen. McHugh *et al.* (2002) were the first to identify the glucocorticoid-induced tumour necrosis factor receptor family-related gene (GITR) as a potential marker of regulatory T cells. Subsequently, it was shown that for the development and function of natural $CD4^+CD25^+$ regulatory T cells, Foxp3, encoding a member of the forkhead transcription factor family, is a 'master control gene'. This showed that abnormality in regulatory T cells can cause human autoimmune disease, allergy and inflammatory bowel disease, as in the human syndrome Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) (Fontenot *et al.*, 2003; Hori *et al.*, 2003; Khattri *et al.*, 2003). Klein *et al.* (2003) were the first to report the dynamics of *in vivo* regulatory T-cell suppression. Dysfunction of a $CD4^+CD25^+$ regulatory T-cell subset has been implicated in the pathogenesis of multiple sclerosis and rheumatoid arthritis (Ehrenstein *et al.*, 2004; Viglietta *et al.*, 2004) and the presence of $CD4^+CD25^+$ regulatory T cells has been associated with improved outcome in lung transplant recipients (Meloni *et al.*, 2004). These observations excited interest in exploring cell-based immunotherapy with $CD4^+CD25^+$ regulatory T cells to treat autoimmunity or promote graft acceptance and tolerance. Battaglia *et al.* (2005) have reported that rapamycin can be used in *ex vivo* expansion protocols to generate sufficient numbers of $CD4^+CD25^+$ regulatory T cells for cell-based immunotherapy. The role of these cells has been described in a number of viral infections, e.g. retro, herpes and picorna viruses (Mills, 2004; Suvas & Rouse, 2005; Vahlenkamp *et al.*, 2005; Schneider-Schaulies & Dittmer, 2006). The present paper reviews our existing knowledge of suppressor/regulatory T cells and its implication in the pathogenesis of severe dengue disease. Due to space constraints only selected works have been cited.

Different types of regulatory T cells

Regulatory T cells are either naturally occurring (developing in the thymus) or the adaptive antigen-specific regulatory T cells that can be induced from naïve $CD4^+CD25^-$ or $CD8^+CD25^-$ T cells in the periphery under the influence of semimature dendritic cells (DCs), IL-10, transforming growth factor-β (TGF-β) and possibly interferon-α (IFN-α) (Bluestone & Abbas, 2003; Mills, 2004). Figure 2

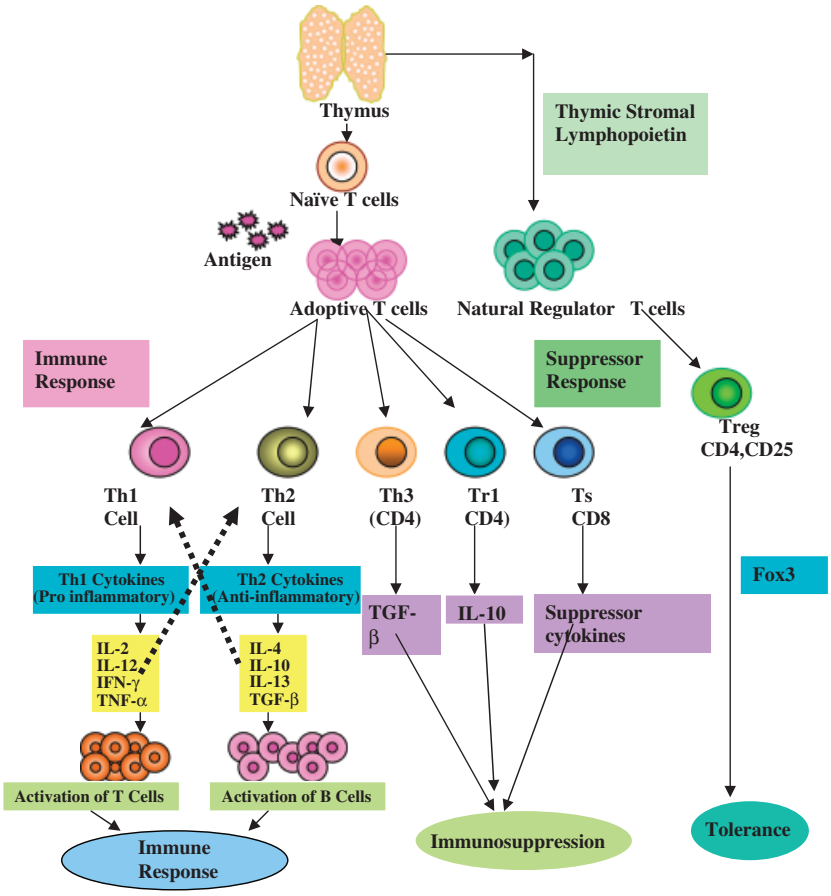

Fig. 2. Generation of immune response and different types of regulatory T cells. A fine balance of interdependent cytokines determines Th1 or Th2 bias in the response. In general, four types of regulatory T cells are generated, natural or antigen-induced.

summarizes the evolution of the immune response and the four major subsets of regulatory T cells.

Natural regulatory T cells

These are recognized by their constitutive expression of CD4 and CD25 and the transcription factor foxP3 and surface CD152. They depend on TGF-β for their generation and the suppressive activity. They can induce indoleamine 2,3-dioxygenase (IDO) in appropriate DCs by CD152-mediated ligation of CD80/86. They are generated in the thymus and represent about 5–10% of $CD4^+CD8^-$ thymocytes in humans and migrate to populate the periphery and do not recirculate (Stassen *et al.*, 2004). The number of Treg cells generated depends on the diversity of major histocompatibility (MHC) class II/peptide complexes in the thymus, leading to the selection of Treg cells with diverse T-cell antigen receptors (TCRs) (Pacholczyk *et al.*, 2002). Recently it has been reported that Hassall's corpuscles present in the thymus produce chemical signals, the thymic stromal lymphopoietin (TSLP), that activates dendritic cells in the thymus to induce development of the Treg cells (Watanabe *et al.*, 2004; Liu *et al.*, 2007). This means that the thymus is providing central tolerance not only through clonal deletion as was previously proposed by the clonal selection theory, but also through clonal conversion. Natural Treg cells appear to play a role in regulating chronic infections, such as HIV and hepatitis C (Belkaid & Rouse, 2005).

Th3 cells

Th3 $CD4^+$ regulatory cells were identified during the course of investigations into the mechanisms associated with oral tolerance. Th3 regulatory cells form a unique T-cell subset that primarily secretes TGF-β, provides help for IgA and has suppressive properties for both Th1 and Th2 cells. Th3-type cells are distinct from Th2 cells, as $CD4^+$ TGF-β-secreting cells with suppressive properties have been generated from IL-4-deficient animals. *In vitro* differentiation of Th3 cells from Th precursors from TCR transgenic mice is enhanced by culture with TGF-β, IL-4, IL-10 and anti-IL-12. Th3 regulatory cells are triggered in an antigen-specific fashion but suppress in an antigen-nonspecific fashion (Weiner, 2001). Recent results of Carrier *et al.* (2007) suggest that TGF-β-derived Foxp3(+)CD25(+/−) Th3 regulatory cells represent a different cell lineage from thymically derived

CD25(+) regulatory T cells in the periphery but may play an important role in maintaining thymic regulatory T cells in the peripheral immune compartment by secretion of TGF-β.

Tr1 cells

This comprises a subset of $CD4^+$ helper T cells that are dependent on IL-10 for their differentiation and their regulatory properties. They secrete high levels of IL-10, no IL-4 and no or low levels of IFN-γ and may express markers associated with Th2 cells and repressor of GATA (ROG) but not fox3. They express high levels of surface CD152 like natural regulatory T cells and can induce IDO and tryptophan catabolism in appropriate DCs (Mills, 2004).

Suppressor T cells

Like Tr1 cells, $CD8^+CD25^-$ suppressor T cells need the presence of IL-10 for induction, and IL-10 may be involved in the downregulation of dendritic cell costimulation and the upregulation of ILT-3 and ILT-4 (in human DCs). IL-10-secreting Tr1 cells have been implicated in the regulation of immune responses elicited by viruses (Iwashiro *et al.*, 2001) and bacteria (McGuirk *et al.*, 2002), etc.

Besides these, some other cells have also been termed regulatory T cells. A subpopulation of 'anergic' $CD4^+$ cells is generated by antigen stimulation without costimulation. They are characterized by an intrinsic increase in their threshold for antigen stimulation, which may be maintained by expression of E3 ubiquitin ligases such as GRAIL, c-cbl and Itch. They adsorb stimulatory cytokines such as IL-2 at the sites of antigen presentation, thus acting as regulatory T cells. The anergic cells differ from the regulatory T cells functionally, in expression of molecular signature and in pathological effecter cell responses (Knoechel *et al.*, 2006). They have been generated *in vivo* during certain infections, e.g. rabies virus (Hirai *et al.*, 1992). Other cells, for example natural killer cells (NKTs) and γδ T cells, have also been categorized as regulatory T cells (Mills, 2004). The distribution and trafficking of regulatory T cells in various tissues depends on distinct chemokine receptors and integrin molecules (Fig. 3).

Dengue virus infection

DV belongs to the family *Flaviviridae* and is transmitted by *Aedes* mosquito, which is found in tropical and subtropical regions around the world, predominantly in urban and semiurban and now in rural areas also. DV has four antigenically related serotypes (1–4) and causes self-limiting milder febrile illness, dengue fever (DF), or a severe disease, dengue haemorrhagic fever (DHF). The main amplifying host of the virus are humans. Infection by one serotype of DV provides long-lasting immunity against the same but confers only partial and transient immunity against infection by the other three serotypes. The prevalence of dengue has grown dramatically and the disease is endemic in more than 100 countries in Africa, the Americas, the eastern Mediterranean, South-East Asia and the western Pacific. South-East Asia and the western Pacific are most seriously affected. Two-fifths of the world's population is now at risk from dengue. The World Health Organization currently estimates there may be 50 million cases of dengue infection worldwide every year and estimated that 500 000 cases of DHF require hospitalization each year, of whom a very large proportion are children (Rigau-Perez *et al.*, 1998; Agarwal *et al.*, 1999a; Malavige *et al.*, 2004). 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 thrombocytopoenia. Extensive plasma leakage in various serous cavities of the body including the pleura, pericardium and peritoneal cavities in DHF may result in profound shock, the dengue shock syndrome (DSS). There is no specific therapy or a vaccine to prevent dengue disease. (Halstead, 1993, 2002; Malavige *et al.*, 2004).

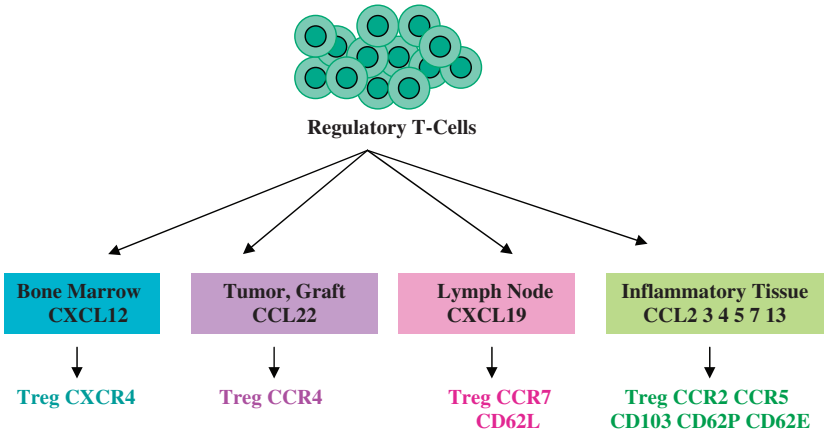

Fig. 3. Distribution of regulatory T cells in various tissues. Distinct chemokine receptors and integrin molecules are implicated in regulatory T-cell organ/tissue trafficking and compartmentalization. Bone marrow-derived CXCL12 mediates Treg-cell bone marrow trafficking. Environmental CCL22 mediates regulatory T-cell trafficking into human ovarian cancer and mouse cardiac grafts. The lymphoid homing molecules CCR7 and CD62L may facilitate lymphoid homing of regulatory T cells. Certain CC chemokines and integrins may mediate regulatory T-cell trafficking into inflammatory tissues/organs (Wei *et al.*, 2006).

Immune response to DV

The first line of the innate host defence against invading DV in skin, where it replicates after the initial bite by an infected mosquito, is provided by the macrophage-lineage cells, interstitial DCs and Langerhan cells (LCs). Early activation of NK cells and type-I IFN-dependent immunity may limit viral replication at the early stages of DV infection (Shresta *et al.*, 2004a).

DV is composed of 10 viral proteins, the core and membrane proteins, the envelope (E) glycoprotein, and seven nonstructural (NS) proteins. Anti-E antibodies inhibit DV binding to cells, neutralize viral infectivity *in vitro*, show a variable degree of cross-reactivity among the DV serotypes and protect mice from DV challenge on passive transfer (Kaufman *et al.*, 1987; Roehrig *et al.*, 1998). The uptake of DV+ nonneutralizing antibody complex into monocytic cell lines and primary human monocytes *in vitro* is enhanced through binding to the Fc-receptor, known as antibody-dependent enhancement of infection (Morens & Halstead, 1990). Complement-mediated lysis of DV-infected cells can be triggered by antibodies against NS1 *in vitro* but they may cross-react with endothelial cells leading to their activation and expression of cytokines, chemokines and adhesion molecules, resulting in cell damage (Schlesinger *et al.*, 1987; Lin *et al.*, 2005). DV-reactive $CD4^+$ and $CD8^+$ T cells are stimulated mainly by NS3 protein, which produces high levels of IFN-γ as well as TNF-α, TNF-β, and chemokines upon interaction with DV-infected antigen presenting cells (APCs), and are efficient at lysis of DV-infected cells *in vitro* (Kurane *et al.*, 1991; Loke *et al.*, 2001).

Role of T cells in dengue

$CD4^+$ T lymphocytes play a central role in regulating the cell-mediated immune response to infection. Some $CD4^+$ T cells can develop into Cytotoxic T lymphocytes (CTL) and can attack B cells, macrophages and DCs (Chinen & Shearer, 2005; Wing *et al.*, 2005). The activated $CD4^+$ T cell is capable of recognizing the antigen and act by releasing cytokines in response. The interaction between antigen-specific $CD4^+$ T cells and macrophages forms the basis of the delayed-type hypersensitivity response, which is one of the main effecter mechanisms in eliminating infections with intracellular organisms (Wing *et al.*, 2005; Huber & Schramm, 2006). The NS3 is an important target of $CD8^+$ T cells in secondary DV infection and the activation and expansion of DV-specific T cells is greater in subjects with DHF than in those with DF, which may play an important role in the pathogenesis of DHF (Simmons *et al.*, 2005). The results highlight the importance of NS3 and cross-reactive T cells during acute secondary infection.

A DV-specific memory lymphocyte response has been detected even 20 years after a primary infection by DV (Sierra *et al.*, 2002). Mongkolsapaya *et al.* (2003) have studied DV-specific T-cell responses in Thai children. During acute infection, few dengue-responsive $CD8^+$ T cells were recovered; most of those present showed an activated phenotype and were undergoing programmed cell death. Many DV-specific T cells were of low affinity for the infecting virus and showed higher affinity for other, probably previously encountered strains. Cross-reactive DV-specific T cells seem to show suboptimal degranulation but high cytokine production, which may contribute to the development of the vascular leak characteristic of DHF (Mongkolsapaya *et al.*, 2006). The prior infection history of the individual as well as the serotypes of the primary and heterologous secondary viruses influences the nature of the secondary response. Profound T-cell activation and death may contribute to the systemic disturbances leading to DHF, and original antigenic sin in the T-cell responses may suppress or delay viral elimination, leading to higher viral loads and increased immunopathology. High levels of T-cell activation, coupled with rapid cell death and the domination of the cellular immune response by cells with a low affinity for the infecting virus, may suppress or delay virus clearance leading to high viral loads and increased immunopathology (Stephenson, 2005).

Immunopathogenesis of DHF

Despite extensive studies, the pathogenesis of DHF is still not fully understood. The mechanisms that have been considered include immune complex disease, antibodies cross-reacting with vascular endothelium, enhancing antibodies, complement and its products, memory T cells, various soluble mediators including cytokines, selection of virulent strains and virus virulence (Halstead, 1993; Chaturvedi *et al.*, 1997, 2000, 2005, 2006a; Mongkolsapaya, 2003; Cologna *et al.*, 2005; Lin *et al.*, 2005). Both viral and host factors influence the disease severity. The risk of severe disease is higher in children, with worse outcome with the Asian strain whereas American strains of DV largely affect the adult population and produce milder disease. This correlates with the structural difference in the two strains of DV and the host genetics (Leitmeyer *et al.*, 1999; Watts *et al.*, 1999; Rico-Hesse, 2003; Cologna *et al.*, 2005; Chaturvedi *et al.*, 2006b).

Disturbed cytokine cascade is the most supported hypothesis (Pang *et al.*, 2007). The key cytokines that have been associated with DHF include the shift from Th1-type response in DF to the Th2-type cytokine response in DHF with increased levels of IL-10 and IL-4 (Chaturvedi *et al.*, 1999). The increased levels of TNF-α (reviewed by Chaturvedi *et al.*, 2000), TGF-β (Agarwal *et al.*, 1999b) and IL-8 (Raghupathy *et al.*, 1998) have been associated with DHF, and IL-12 with DF (Pacsa *et al.*, 2000). Patients with defects in the IL-12 receptor or IFN-γ receptor have abnormal

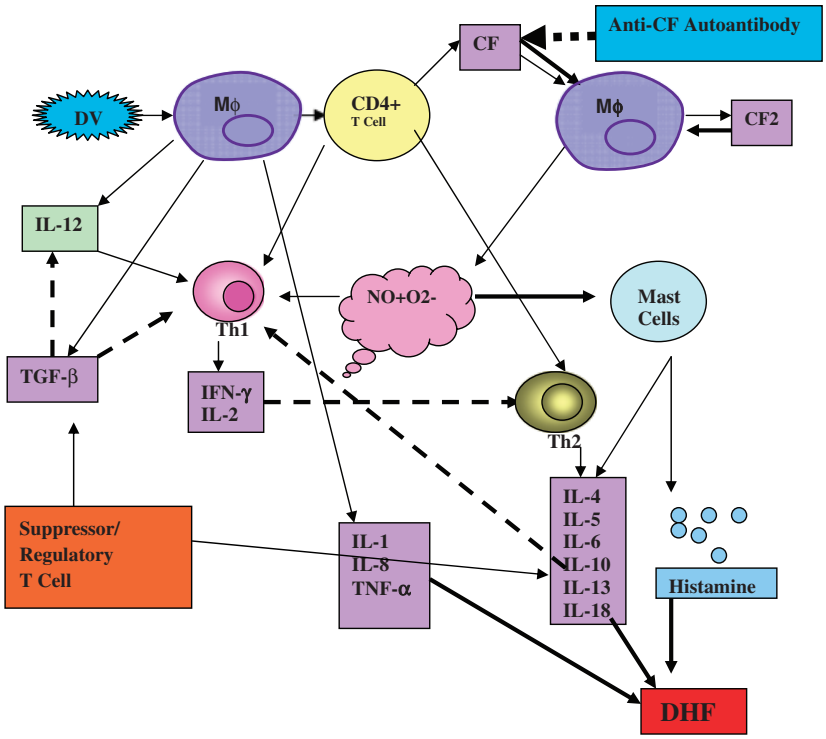

Fig. 4. Interaction of various cytokines/secretary products during dengue virus infection. Any shift in the bias towards the Th2 response precipitates dengue haemorrhagic fever (DHF). Thin lines, positive induction; thick lines, damaging effect; dashed lines, inhibition.

responses to IL-12 or IFN-γ and a failure to produce normal levels of IFN-γ (Catherinot *et al.*, 2005; Wood *et al.*, 2005).

We have proposed a mechanism that may explain the pathogenesis of DHF (Chaturvedi *et al.*, 2000). DV replicates in macrophage-like cells and rapidly induces the $CD4^+$ T cells to produce a unique cytokine, the cytotoxic factor (hCF) (Agarwal *et al.*, 1998). hCF induces macrophages to produce free radicals, nitrite, reactive oxygen and peroxynitrite (Misra *et al.*, 1996, 1998). The free radicals, besides killing the target cells via apoptosis, also directly upregulate production of proinflammatory cytokines IL-1, TNF-α, IL-8 and hydrogen peroxide in macrophages. 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 the patients. Vascular permeability is increased due to the combined effect of histamine, free radicals, proinflammatory cytokines and the products of the complement pathway, etc (Fig. 4). Thus, the key player appears to be hCF. Furthermore, the elevated levels of hCF autoantibodies protect the patients against the development of severe forms of DHF and therefore it may be useful as a prognostic indicator (Chaturvedi *et al.*, 2001).

Among the several hypotheses proposed to explain the pathogenesis of severe dengue disease, two are from landmark studies. First was the role of enhancing antibodies (Halstead, 1970) and the second was the demonstration of a shift from a Th1 response in mild dengue to a Th2-response in severe DHF (Chaturvedi *et al.*, 1999) associated with disturbance to the fine balance of the cytokine cascade, resulting in a 'cytokine storm', which causes all the pathological lesions (Chaturvedi *et al.*, 2000). Given the severity and speed with which the clinical condition of the patient deteriorates, with multiorgan involvement, we prefer the term 'cytokine tsunami', i.e. it washes away every thing in its tide.

Suppressor T cells in dengue

DV was the first infectious agent against which TS cells were demonstrated and used to delineate the sequential events in the suppressor pathway that has been presented here as a model of the chain of events in an infection.

Induction of suppressor T cells

In DV infection of mice, the virus replicates in the spleen and other organs (Chaturvedi *et al.*, 1978), thus overloading with antigen; the Macrophage (Mϕ) are killed or become functionally detective (Chaturvedi *et al.*, 1982, 1983; Gulati *et al.*, 1982), and the presence of immune complexes have been shown in human infection. Therefore, the above factors, singly or combined in various proportions, provide a strong stimulus for the generation of suppressor cells. DV induces suppressor cells both by i.p. or i.c. routes and activity lasts for 3 weeks (Shukla & Chaturvedi, 1985). The phenotype of DV-induced TS1 and TS2 cells is $CD8^+$ (mouse = $Ly23^+$) and that of TS3 cells is $CD4^+$. The inducer

cells of suppressor activity are $CD4^+$ T cells. In the DV model, TS1 and TS2 appear to be suppressor cells while the TS3 cells appear to induce suppression of B-cell activity (Chaturvedi, 1984).

Production of soluble suppressor factors

TS cells may mediate their activity either through direct contact of interacting cells or through elaboration of soluble factors. In the DV model the TS1 cells produce a soluble suppressor factor (SF1) that can be extracted from cells or is secreted in culture fluid (Shukla & Chaturvedi, 1981, 1984). SF1 induces another subpopulation of T cells *in vivo* and *in vitro* to produce another soluble suppressor factor (SF2) that is prostaglandin-like (Chaturvedi & Shukla, 1981; Chaturvedi *et al.*, 1981).

Suppressor T cell cascade

DV-infected mice develop DV antigen-specific immunosuppression, which has been shown to be mediated by a cascade of three generations of TS cells and their secretary soluble suppressor cytokines (SF) with Mϕ transmitting the signals in between (Fig. 5). DV-infected Mϕ transmit the signal to recruit TS1 cells, which secrete a suppressor cytokine, SF1. The suppressor signal of SF1 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). Prostaglandin-producing suppressor cells have also been shown in hepatitis C virus (HCV) patients (Marinho *et al.*, 2002). SF2 induces production of a third subpopulation of suppressor T cells (TS3), which suppresses the humoral immune response in an antigen-specific and genetically restricted manner (Shukla & Chaturvedi, 1981, 1982, 1983, 1984, 1985; Chaturvedi, 1984; Chaturvedi *et al.*, 1985a, b). A few reports show that regulatory T cells are specific for pathogen-derived antigens, e.g. mouse and humans infected with JEV (Mathur *et al.*, 1984, 1987a, b, 1991) and humans infected with HCV and other viruses (Mills, 2004). The effects of prostaglandin E2 (PGE2) on Ig production consist of its indirect effects through T cells and its direct effects on B cells. The outcome of the effects can be upregulatory or downregulatory, depending on whether resistant or sensitive T cells are involved (He *et al.*, 2002). COX-2-mediated PGE2 upregulates IL-10, which downregulates IL-12 production and the APC function of bone-marrow DCs (Harizi *et al.*, 2002). PGE1 inhibits all the adhesion molecule expression, cytokine production and T-cell proliferation in the presence of IL-18 (Takahashi *et al.*, 2005). Recently it has been reported

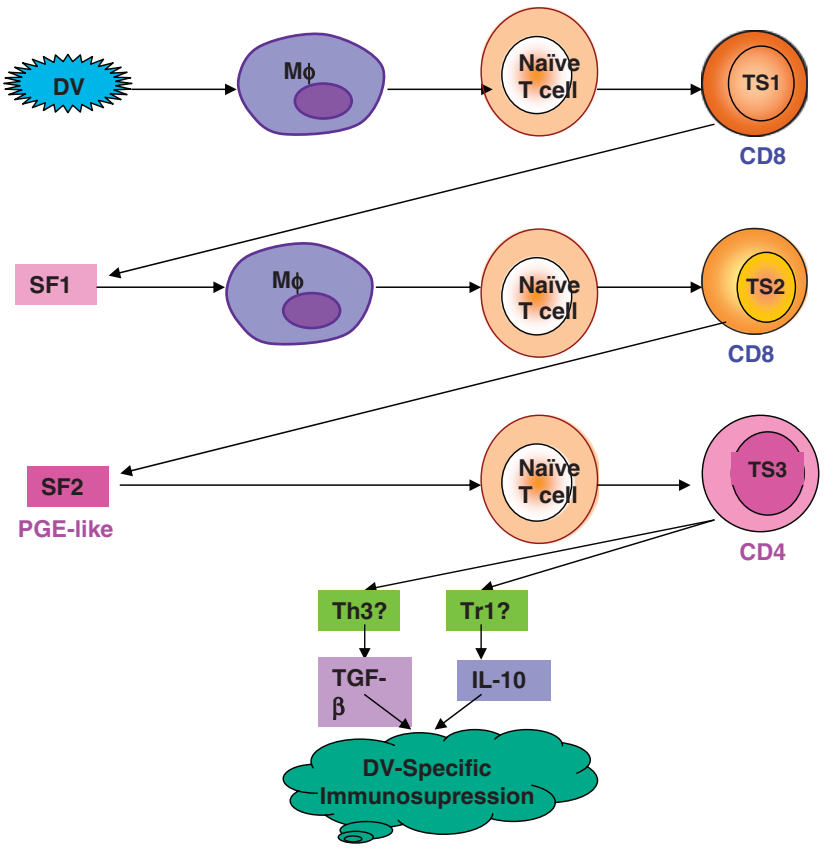

Fig. 5. Dengue virus (DV) induces a suppressor pathway in mice which involves sequential production of three generations of suppressor T cells (TS1, TS2, TS3). TS1 cells produce a soluble suppressor factor (SF1), which transmits the signal via macrophages to generate TS2 cells, which produce a prostaglandin-like soluble suppressor factor (SF2) to recruit TS3 cells, which suppress the antigen-specific immune response. It is not known whether TS3 is Tr1 or Th3 or is a separate type of regulatory T cell. The properties of these suppressor cells and their products have been discussed as a model in relation to pathogenesis of the disease. It is concluded that the generation of the suppressor pathway is a protective as well as destructive phenomenon.

Table 1. Receptors on various cells for DV-induced suppressor factor

Cell	No. of receptors/ cell (affinity)	No. of polypeptide chains in cytokine	No. of polypeptide chains in receptor	Sites of binding of cytokine on cells	References
Macrophage	M: 54 000 (HA) M: 1.78×10^6 (LA)	α chain β chain	α chain β chain	SFα: SFRβ SFβ: H-2A	Mukherjee *et al*. (1993a, b, 1994), Tripathi *et al*. (1997)
T cell	T: 35 000 (HA) T: 0.72×10^6 (LA)				
B cell	B: 16 000 (HA) B: 0.33×10^6 (LA)				

M, macrophage; T, T lymphocyte; B, B lymphocyte; HA, high-affinity receptor; LA, low-affinity receptor; SFR, receptor for SF.

that in resting $CD4^+CD25^-$ T cells, treatment with PGE2 induces FOXP3 expression, which may be the mechanism used by these cells to suppress effecter T cells (Baratelli *et al*., 2005; Mahic *et al*., 2006).

Role of macrophages in transmission of DV-specific cytokine signal

Cytokines transmit their signal via receptors on target cells. The receptors studied for DV-induced SF1 are presented in Table 1. The study undertaken to investigate the intermediary role of Mϕ in transmission of signal from TS1 to TS2 showed that live syngeneic macrophages adsorb SF1 and transmit the signal to naive T cells to recruit TS2. This is not possible with killed Mϕ or in the absence of live Mϕ (Shukla & Chaturvedi, 1982). Furthermore, transmission of suppressor signal from SF1-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 (Shukla & Chaturvedi, 1983). The suppressive function of natural and inducible regulatory T cells has been shown to be mediated either through secretion of immunosuppressive cytokines or through cell–cell contact (Mills, 2004). Pretreatment of Mϕ with the calcium channel blockers that block the influx of calcium inhibit transmission of the suppressor signal from TS1 to TS2 cells in a dose-dependent manner. The presence of calcium ions is obligatory for transmission of the suppressor signal (Khare & Chaturvedi, 1995). Similarly, nitrous oxide also serves as an intracellular signal in the transmission of suppressor signal in Mϕ (Khare & Chaturvedi, 1997).

SF1 is composed of two polypeptide chains (α and β) (Bhargava *et al*., 1990). 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 composed of two polypeptide chains (α and β), which are obtained in pure form by HPLC of dithiothreitol- and iodoacetamide-treated SF-R (Mukherjee *et al*., 1993a). Both high- and low-affinity receptors are present on T and B cells. Both the α- and the β-chains of SF purified by HPLC bind to Mϕ, but only the α-chain binds to SF-R protein while the β-chain of SF binds to H-2A determinants on Mϕ (Mukherjee *et al*., 1993b, 1994). SF binds to both high- and low-affinity SF-R on Mϕ and that bound to high-affinity receptors is internalized through receptor-mediated endocytosis. Pretreatment 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 the 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 (Fig. 6). Thus, SF is internalized, degraded and binds to H-2K antigen before its recognition by native T cells (Tripathi *et al*., 1997).

Role of TS cells in the immunopathogenesis of DHF

Regulatory T cells are a double-edged sword. They are beneficial to the host by preventing immunopathology and help in the development of immune memory. On the other hand, they help the pathogen to establish a chronic infection. TS cells, including memory TS cells, have been shown to play an important role in the continued battle between the virus and immunological memory cells, resulting in latency and persistence of viruses. JEV is known to produce a persistent and latent infection in mice (Mathur *et al*., 1986a, b) and human patients (Mathur *et al*., 1991) that can be reactivated. Immunological memory is retained in the latent JEV infection of mice (Kulshreshtha *et al*., 1988) and the reactivated JEV in latently infected mice induces a secondary immune response (Mathur *et al*., 1987a). The presence of memory TS cells in mice latently infected with JEV has been shown, and these can be stimulated to generate secondary TS cells by exogenous or endogenous virus challenge (Mathur *et al*., 1987b). Sheridan *et al*. (2006) have reported that latent virus influences the generation and maintenance of CD^+ T-cell memory. This phenomenon could help in the persistence of the virus.

Early activation of NK cells and type-I IFN-dependent immunity may limit viral replication at the early stages of DV infection. IFN-α/β is critical for early immune responses

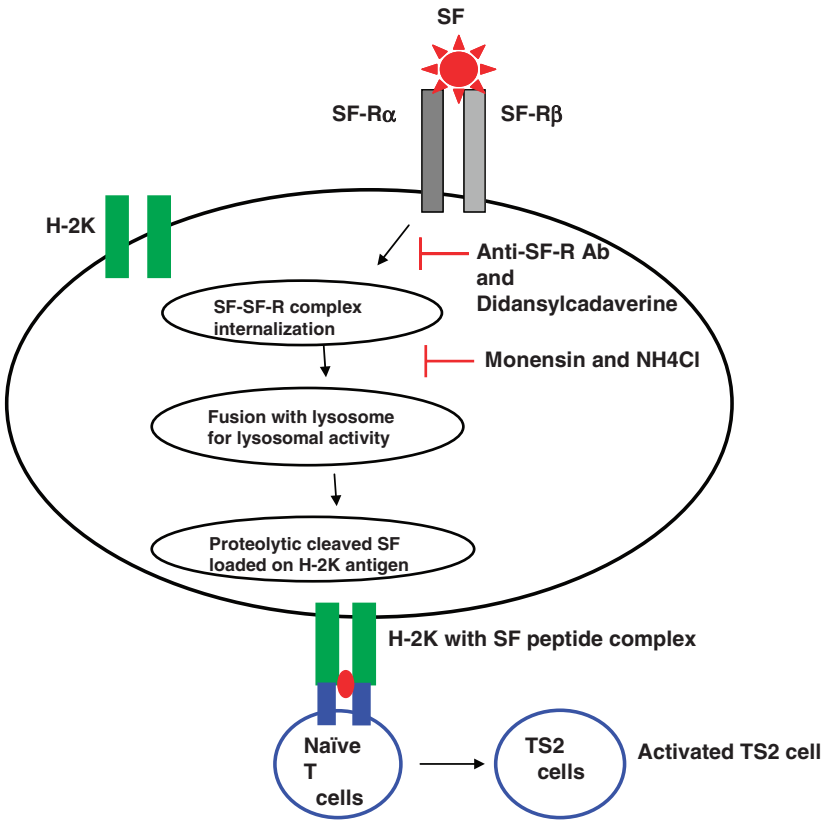

Fig. 6. Internalization of suppressor factor (SF) through suppressor factor receptor (SF-R) expressed on macrophages after DV infection: SF binds to SF-R on macrophages and complex is internalized into coated pits that enclosed the vesicular compartment. The SF-SF-R complex required for initiating suppressor activity is inhibited by anti-SF-R-Ab and didansylcadaverin, which is an inhibitor for receptor-mediated internalization. The vesicular compartment loaded with complex is fused with lysosomes for cleavage of SF into peptides. The lysosomal activity of lysosomes is inhibited by monensin and NH_4Cl. The proteolytically cleaved SF is loaded on H-2K antigen and the complex is transported on the cell surface. The complex is recognized by TS2 cells and is activated. The activation of TS2 cells is inhibited by anti-SF-Ab but not by anti-H-2K-Ab.

to DV infection while IFN-γ-mediated immune responses are crucial for both early and late clearance of DV infection in mice (Shresta *et al.*, 2004a). Early activities of NK cells, B cells and IgM, and later actions of IFN-γ and IgG are likely to play a role in the defence against DV infection (Shresta *et al.*, 2004b). Failure of NK cells to contain DV replication may result in severe DHF/DSS, which is associated with Th2-type response (Chaturvedi *et al.*, 1999, 2000, 2006a, b). A similar enhancement of Th-2-type response and severe disease has been reported in *Bordetella pertussis* infection in the absence of NK cells (Byrne *et al.*, 2004). Histamine may mediate deleterious effects on vascular permeability in patients with DHF (Chaturvedi *et al.*, 2000) and can also modulate the Th1/Th2 cell balance.

A balanced Th1 and Th2 response controls the immune response to microorganisms. Severe dengue disease is associated with a predominant Th2 response and markedly increased levels of a number of cytokines, including IL-10 and TGF-β (Table 2). PGE is known to enhance the levels of intracellular cAMP, whichblocks the development of IL-12 responses (Van der Pouw Kraan *et al.*, 1995). SF2 is a PGE-like molecule (Chaturvedi *et al.*, 1981; Shukla & Chaturvedi, 1981), and may inhibit IL-12 production in DV infection. By suppressing protective Th1 responses the regulatory T cells may enhance the Th2 response and increase the infection-induced immunopathology. Antigen-specific Tr1 or Th3 cells secrete IL-10 and/or TGF-β, but no IL-4 and little or no IFN-γ, and are induced by semimature dendritic cells under the influence of regulatory cytokines, including IL-10, TGF-β and IL-4. Tr1 or Th3 cells are capable of suppressing Th1 or Th2 responses and functions in infection (Mills & McGuirk, 2004). Loss of suppressor of cytokine signalling (SOCS3) in T helper cells results in reduced immune responses and hyperproduction of IL-10 and TGF-β (Kinjyo *et al.*, 2006). Suppression of Th1 response by Tr1 or Th3 cells in DV infection may result in pathogen-induced immunopathology. It is not known whether the TS3 cells described in DV infection are Tr1 or Th3 or a separate lineage. McGuirk & Mills (2002) have shown that pathogen-specific regulatory T cells provoke a shift in the Th1/Th2 paradigm in immunity to infectious diseases. Is this true in DHF also where a shift to Th2 response results in severe disease. Both the regulatory T cells and Th2 cells secrete IL-10 and TGF-β, thus disturbing the fine balance in the different cytokines and causing a 'cytokine tsunami' and severe dengue disease.

The DV-induced suppressor pathway suppresses antigen-specific antibody production, including that of the enhancing antibody. Thus, increased replication of the virus mediated by the enhancing antibody and the immunopathology mediated by the immune complex is prevented.

Table 2. Cytokine profile in patients with dengue

Cytokine	Dengue fever	Dengue haemorrhagic fever	References
Interleukin-2	Marked increase	Increased	Kurane *et al*. (1991), Chaturvedi *et al*. (1999)
Interleukin-4	Increased	Marked increase	Chaturvedi *et al*. (1999)
Interleukin-6	Increased	Marked increase	Hober *et al*. (1993), Chaturvedi *et al*. (1999)
Interleukin-8	Decreased	Marked increase	Raghupathy *et al*. (1998)
Interleukin-10	Decreased	Marked increase	Chaturvedi *et al*. (1999)
Interleukin-12	Marked increase	Decreased	Pacsa *et al*. (2000)
Interleukin-13	Increased	Marked increase	Mustafa *et al*. (2001)
Interleukin-18	Increased	Marked increase	Mustafa *et al*. (2001, p. 58)
Interferon-γ	Marked increase	Increased	Kurane *et al*. (1991), Chaturvedi *et al*. (1999)
Tumour necrosis factor-α	Marked increase	Marked increase	Hober *et al*. (1993), Chaturvedi *et al*. (1999)
Transforming growth factor-β	Decreased	Marked increase	Agarwal *et al*. (1999b)
Cytotoxic factor	Increased	Marked increase	Agarwal *et al*. (1998)

Mainly cited are those references which reported for the first time in human patients. These results have been confirmed in subsequent studies by different groups.

Table 3. Effects of suppressor T cells on the severity of dengue disease

Functions of suppressor T cell	Effects of suppressor T-cell activity	Effects on disease severity	References
Suppression of enhancing antibody	Depressed opsonization of DV	Protected	Chaturvedi (1984)
Suppression of neutralizing antibody	Depressed neutralization of DV	Increased	Chaturvedi (1984)
Suppression of hCF-autoantibody	Increased production of hCF	Increased	Chaturvedi *et al*. (2001)
Suppression of cell-mediated immunity (DTH)	Depressed opsonization of DV	Increased/Protected	Chaturvedi *et al*. (1978, 1884)
Suppression of Th1 response	Depressed production of IFN-γ, IL-2	Increased	Chaturvedi *et al*. (2000)
Suppression of Th2 response	Depressed production of IL-10, TGF-β	Protective	Chaturvedi *et al*. (2000)
Suppression of hCF-producing CD4 T cell	Depressed production of hCF	Protective	Chaturvedi *et al*. (2000)
Suppression of IL-12	Depressed Th1 response	Increased	Chaturvedi *et al*. (2000)
Increased production of SF2 (prostaglandin-like) by TS2	Increased inflammatory response, body pain	Increased	Shukla & Chaturvedi (1981), Chaturvedi *et al*. (1981), Shukla & Chaturvedi (1981)
Increased production of nitric oxide by Mϕ exposed to SF1	Increased inflammatory response	Increased	Khare & Chaturvedi (1997)
*Increased production of IL-10 by TS3?	Increased inflammatory response	Increased	Mills (2004)
*Increased production of TGF-β by TS3?	Increased inflammatory response	Increased	Weiner (2001)
Increased phagocytosis by Mϕ-like cells	Increased replication of DV	Increased	Nagar *et al*. (1985)

A cascade of cytokines produces the complex phenomenon of 'cytokine tsunami' that damages tissues, and absence of it helps in recovery from immunopathology. No single cytokine can mediate this complex process.
*Tr1 cells produce IL-10 and Th3 produce TGF-β. DV-induced TS3 may be Tr1 or Th3 or neither of them.

On the other hand, suppression of neutralizing antibody would delay elimination of DV from the body causing pathological lesions (Chaturvedi, 1984; Chaturvedi *et al*., 2006a). Furthermore, in certain strains of mice Delayed type hypersensitivity (DTH) can be induced (Pang *et al*., 1982) while in others this is not possible (Chaturvedi *et al*., 1977, 1978). In 'nonresponder' strains where the immune response does not develop against an antigen the role of suppressor cells has been suggested (Chaturvedi, 1984). In primed mice DV-induced suppressor cells last for 3 weeks and on adoptive transfer the suppressor activity of TS cells persists for 3 weeks in recipient mice and that of SF1, TS2 and SF2 lasts 1–2 weeks (Shukla & Chaturvedi, 1985). Suppressor T-cell activity, regulating B- and T-cell responses in dengue type 3 virus-infected mice has been shown (Nagarkatti & Nagarkatti, 1983). Induction of suppressor cells in DV may suppress the cell-mediated immune response and thus protect the body against T-cell-mediated damage. Recently, the influence of human genes on DF and DHF has been discussed revealing associations between Human leucocyte antigen (HLA) polymorphism and dengue disease susceptibility or resistance, protective alleles influencing progression to severe disease, and alleles restricting $CD4^+$ and $CD8^+$ T-cell and non-HLA genetic factors that may contribute to DHF evolution, e.g. genes influencing production of various cytokines (Chaturvedi *et al*., 2006b).

This may also have a bearing on production of regulatory T cells in DV infection. The role of suppressor/regulatory T cells in dengue disease is summarized in Table 3.

Conclusions

Suppressor/regulatory T cells appear to play a key role in the immunopathogenesis of severe dengue disease (DHF). The biological significance of a multistep pathway is in self-regulation and amplification of the signal. The product of one T cell can activate more than 100 cells, thus amplifying the signal. The data from mouse model cannot be extrapolated to humans but they strongly indicate the possibility of the existence of such a mechanism in humans that determines the outcome of infection. There is a need to investigate human cases of dengue infection on similar lines using modern technology to identify markers on suppressor T-cell subpopulations and characterize the suppressor factors. The paradigm of a shift from Th1 to Th2 cells in patients with DHF needs re-examination in view of the suggested role of the Th17 cell pathway (Steinman, 2007). The future may see manipulation of the regulatory T-cell subset for use in patients to treat pathological inflammatory responses by enhancing/depressing their suppressive function. The suppression or depletion of regulatory T cells may be employed to improve response to dengue vaccines. The precise mechanisms that control regulatory T cells *in vivo* are not clear. Several established immunomodulatory treatments may influence regulatory T cells. In view of the fine balance between upregulation and downregulation of cytokine responses in dengue, as discussed above, the modification of regulatory T cells *in vivo* will have to be approached with caution to prevent any unwanted side-effects. Further investigation into the development of new drugs that enhance/depress the growth of regulatory T cells is crucial.

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