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MiniReview

Cytokine cascade in dengue hemorrhagic fever: implications for pathogenesis

U.C. Chaturvedi [a,*], R. Agarwal [b], E.A. Elbishbishi [a], A.S. Mustafa [a]

[a] *Department of Microbiology, Faculty of Medicine, Kuwait University, P.O. Box 24923, Safat 13110, Kuwait*
[b] *Department of Microbiology, Sanjay Gandhi P.G.I.M.S., Lucknow 226014, India*

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Abstract

Dengue virus produces a mild acute febrile illness, dengue fever (DF) and a severe illness, dengue hemorrhagic fever (DHF). The characteristic feature of DHF is increased capillary permeability leading to extensive plasma leakage in serous cavities resulting in shock. The pathogenesis of DHF is not fully understood. This paper presents a cascade of cytokines, that in our view, may lead to DHF. The main feature is the early generation of a unique cytokine, human cytotoxic factor (hCF) that initiates a series of events leading to a shift from Th1-type response in mild illness to a Th2-type response resulting in severe DHF. The shift from Th1 to Th2 is regulated by the relative levels of interferon-gamma and interleukin (IL)-10 and between IL-12 and transforming growth factor-β, which showed an inverse relationship in patients with DF. © 2000 Published by Elsevier Science B.V. on behalf of the Federation of European Microbiological Societies.

Keywords: Cytokine; Dengue; DHF; Pathogenesis; Th1; Th2; TNF-α; IFN-γ; TGF-b; IL-1b; IL-2; IL-4; IL-6; IL-8; IL-10; IL-12; IL-13; IL-18; Cytotoxic factor

1. Introduction

Dengue virus (DV) is transmitted through mosquito bite and is prevalent in over 100 tropical and subtropical countries with about 2 000 000 000 people at risk [1]. DV infection is mostly asymptomatic or produces a mild self-limiting acute febrile illness, dengue fever (DF). It can also produce a life threatening severe illness, dengue hemorrhagic fever (DHF) with minor or major bleeding from different sites [2]. DHF has emerged as the most important arbovirus disease in man in the last two decades. It has been estimated that about 50–100 000 000 cases of DF occur every year with about 250 000 to 500 000 cases of DHF [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 pathognomonic features of DHF are increased capillary permeability without morphological damage to the capillary endothelium (only the cell junctions are opened up), altered number and functions of leucocytes, increased hematocrit and thrombocytopenia [4–6]. Extensive plasma leakage in various serous cavities of the body including the pleural, pericardial and peritoneal cavities in DHF grades III and IV may result in profound shock, the dengue shock syndrome (DSS). Despite extensive studies, the pathogenesis of DHF is still not fully understood. Various mechanisms that have been considered include, immune complex disease, enhancing antibodies, complement and its products, various soluble mediators including cytokines and virus virulence etc. (reviewed in [6–8]). In this paper we present a brief review of the cytokines that have been studied in patients with dengue disease and have organized them in a cascade to explain our view on the pathogenesis of DHF.

2. Th1 and Th2 cytokines in patients with dengue

Cytokine secretion profiles distinguish helper T (Th) 1 and Th2 cells, which are the major subsets of fully, differentiated $CD4^+$ Th cells. Th1 cells secrete interferon-gamma (IFN-γ), interleukin-2 (IL-2) and tumor necrosis factor-β (TNF-β) and are responsible for cell-mediated inflammatory reactions, delayed type hypersensitivity, tis-

* Corresponding author. Tel.: +965 (531) 2300 ext. 6560; Fax: +965 (533) 2719/531-8454; E-mail: chaturvedi@hsc.kuniv.edu.kw

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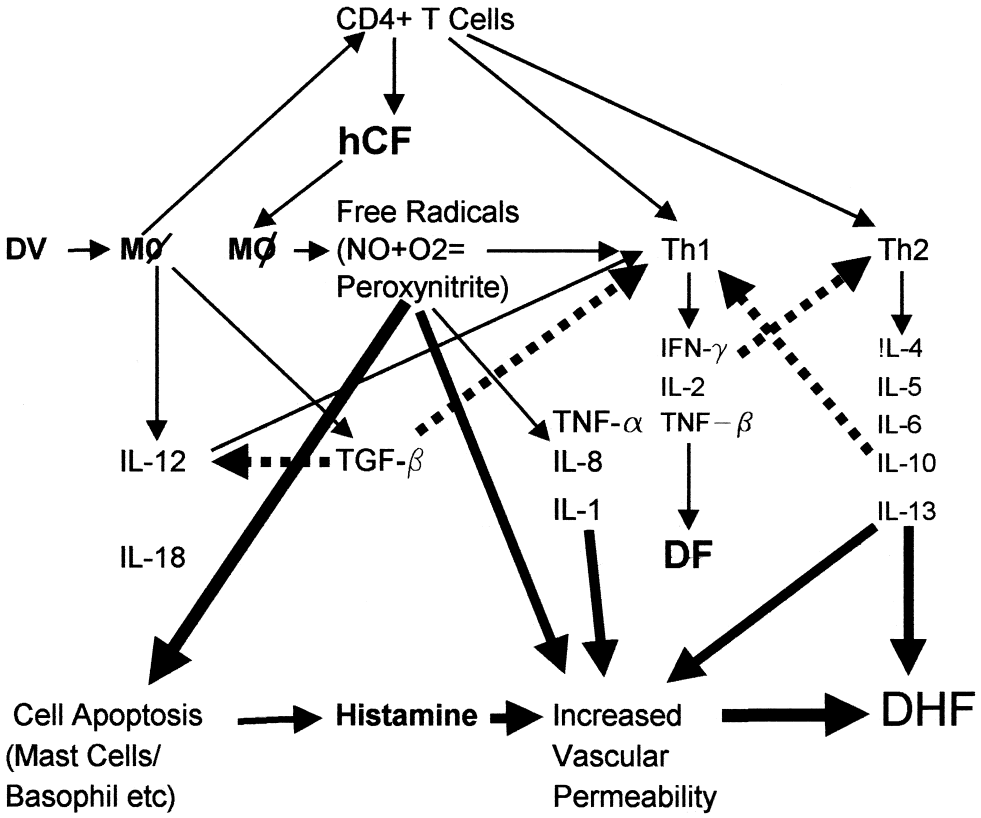

Fig. 1. DV-induced cytokine cascade. DV replicates in macrophage and is presented to recruit $CD4^+$ cells which produce hCF. hCF induces a cytokine cascade that may lead to Th1-type response causing a mild illness, the DF or to a Th2-type response resulting in various grades of severe illness, the DHF. Thin line, positive induction; Interrupted line, inhibition; Thick line, damaging effect.

sue injury in infections and autoimmune diseases. Th2 cells secrete IL-4, IL-5, IL-6, IL-10, and IL-13 (Fig. 1) and are associated with help for B cell antibody production. Cross-regulation of Th1 and Th2 is primarily mediated by IL-10 and IFN-γ respectively (Fig. 1). Infections eliciting a dominant humoral immune response induce a higher expression of Th2-related cytokines, whereas those characterized by delayed-type hypersensitivity response show a higher expression of Th1 cytokines. In a number of parasitic, fungal, bacterial and viral infections such as human immunodeficiency virus, herpes simplex and influenza viruses, a Th1 response is linked to recovery from infection while a Th2-type response tends to lead to severe pathology and exacerbation of the disease (reviewed in [9]).

With regard to dengue disease, the most significant finding of our recent study is a shift from the predominant Th1-type response observed in cases of DF to the Th2-type in severe cases of DHF grade IV (Table 1). Increased serum levels of IL-4, IL-6 and IL-10 were observed mainly in cases of DHF grades III and IV. In contrast, the levels of IFN-γ and IL-2 were highest in cases of DF and low in DHF grade IV. TNF-α levels did not show a definite associative pattern with DF or DHF. The cytokine levels to increase first were IL-2, IL-6, IFN-γ and TNF-α while IL-4, and IL-10 tended to emerge during days 4–8 of the illness [10]. The data were analyzed to ascertain whether the cytokine response in individual patients could be categorized as Th1-biased, Th2-biased or indeterminate (i.e. having a mixed response). The cytokine profile of individual patients with DF showed increased levels of IFN-γ and IL-2 and absence of IL-4, IL-6 and IL-10, a typical Th1-type response while that of DHF grade IV showed increased levels of IL-4, IL-6 and IL-10 and lower levels or absence of IL-2 and IFN-γ, a typical Th2-type response (Table 1). An analysis of total cases showed that 66% of the cases of DF had Th1-type response. As the severity of the illness increased, the response shifted to Th2-type in 71% of the cases of DHF grade IV [10]. The serum levels of IL-13 followed the typical pattern of Th2-type cytokines, being absent in patients with DF and the highest levels in cases of DHF grade IV (unpublished results).

In a follow-up study, we investigated the sequence of appearance of Th1- and Th2-type cytokines in human peripheral blood leucocytes of healthy donors infected in vitro with DV [11]. The cytokines that appeared in the culture supernatants on the first day post-infection (p.i.) were human cytotoxic factor (hCF), TNF-α, IL-2 and IL-6; their levels reached a peak on the second day. IFN-γ appeared on the 2nd day with a peak on the 3rd day. The levels of these cytokines declined quickly except hCF and IL-2. The cytokines that appeared later were IL-10 and

IL-5 on the 4th day and IL-4 on the 6th day p.i. DV replicated in the peripheral blood leucocyte cultures and was present throughout the course of the study. The findings of this study showed that DV induced a predominant Th1-type cytokine response during the first three days of infection of peripheral blood leucocytes in vitro which was replaced by a Th2-type response later [11].

3. Proinflammatory cytokine in patients with dengue

Non-Th1 and Th2 cytokines include IL-1, IL-6 and TNF-α. They play a significant role in the development of acute inflammatory responses. The levels of IL-1β have been studied in patients with dengue (Table 1) and were found to be similar to those of normal healthy controls [12–16]. Although higher levels of TNF-α and IL-6 have been reported in patients with dengue (Table 1) a definite association with the severity of illness was not found [10,12–14,17–20].

4. Macrophage cytokines in patients with dengue

4.1. IL-8:

Elevated levels of IL-8 have been shown in several viral infections, including dengue [21]. We have shown that increased levels of IL-8 in the sera and IL-8-mRNA in the PBMC are associated with the increasing severity of DHF. About half of the patients of DHF grade IV who died had serum levels of IL-8 above 200 pg ml^{-1}, the highest being 5568 pg ml^{-1} in one of them. These results suggest that IL-8 might have an important role in increasing the severity of the disease and death [21]. The intravascular coagulopathy seen in DHF [4] may be attributable, at least in part, to the effects of IL-8. High local IL-8 levels are also associated with pleural effusion [22]. It is tempting to associate the increased vascular permeability with leakage of plasma in DHF with the presence of high levels of IL-8, and it may also be a useful indicator of serious outcome of DF.

Table 1
Cytokines levels in patients with dengue

Cytokines	DF	DHF	References
IL-1β	→	→	[12–16]
IL-2	↑↑	↑	[10,43]
IL-4	↓	↑↑	[10]
IL-6	↑	↑↑	[10,12–14,16]
IL-8	↓	↑↑	[20,44]
IL-10	↓	↑↑	[10,45]
IL-12	↑↑	↓	[25]
IL-13	↓	↑↑	Personal unpublished data
IL-18	↑	↑↑	Personal unpublished data
TNF-α	↑↑	↑↑	[10,12,16–20]
IFN-γ	↑↑	↑	[10,43]
TGF-β	↓	↑↑	[15,29]
hCF	↑	↑↑	[30,31,32,36]

→, no change; ↓, decrease; ↑, increase; ↑↑, marked increase.

4.2. IL-12

IL-12 has a profound effect on the upregulation of Th1 cells and Th1-type cytokines, 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 (reviewed in [23,24]). Elevated levels of IL-12 were seen in the patients with milder DF and complete absence in the patients with DHF grades III and IV (Table 1) [25]. Thus IL-12 might play a role in preventing the severe dengue disease by maintaining the Th1-type response. If this is true, IL-12 therapy could have a profound beneficial effect on the outcome of severe dengue disease as is seen in a number of other viral infections (reviewed in [24]).

4.3. IL-18:

IL-18 was first identified as a costimulatory factor for the production of IFN-γ following endotoxin shock. It is produced by activated macrophages and a variety of other cells. It has structural homology with the IL-1 family and shares with IL-12 the function of inducing Th1 cells to produce IFN-γ (reviewed in [26]). In dengue, IL-18 was present in 40% of the patients with mild illness. The positivity increased with the increasing severity of the illness and was highest (80%) in patients with DHF grade IV (unpublished results). This is unlike IL-12, which was high in DF and absent in patients with DHF grades III and IV and is contrary to expectation, as all the Th2-type cytokines are high in DHF grade IV patients (Table 1). A recent study has shown that IL-18 is a potent co-inducer of IL-13 in NK and T cells, and depending upon the cell type, it can act as a strong coinducer of Th1 or Th2 cytokines [27]. This could explain its role in dengue.

4.4. Transforming growth factor-β1 (TGF-β1)

TGF-β has multiple immunomodulatory effects on various target cells. TGF-β1 may act as a proinflammatory or anti-inflammatory cytokine depending upon its concentration. In the acute phase it induces secretion of IL-1α and TNF-α to control the infection; however, TGF-β also decreases the production of free radicals, inhibits receptor expression and functions of IFN-γ, IL-1α, IL-2 and TNF-α, inhibiting Th1-type cytokines and enhancing production of Th2-type cytokines such as IL-10 (reviewed in [28]). In patients infected with DV, both the severity of disease and the duration of illness were correlated with the level of TGF-β1, i.e., the maximum levels of TGF-β1 were detected in patients with DHF grade IV (Table 1) and those who have the disease for more than 9 days [29].

The only other cytokine to show such high positivity in patients with dengue is hCF which is also associated with the severity of the illness. [5,10,30–32]. The results of serum levels of TGF-β1 and IL-12, when analyzed together, showed an inverse relationship in patients with DF and with various grades of DHF [25].

5. hCF in patients with dengue

The macrophage is the principal cell to replicate DV and is obligatory for the presentation of its antigen [34,35]. This recruits $CD4^+$ cells that produce a unique cytokine, CF, in mice (mCF) and in man (hCF). The amino-terminal sequence of mCF has no homology with any of the known proteins or cytokines [5,36]. The hCF purified from the sera of DHF patients, when inoculated into mice increased capillary permeability and damage to the blood-brain barrier indicating its role in pathogenicity [36]. mCF and hCF appear to be pathogenesis-related proteins, capable of reproducing DHF-like pathological lesions in mice, such as increased capillary permeability, cerebral edema, and blood leukocyte changes (reviewed in [5,36,37]). During an extensive epidemic of DHF in Northern India during 1996, the presence of hCF was shown in 90% of the 333 cases with peak amounts in the most severe cases of DHF grade IV [11]. Further, ex vivo culturing of peripheral blood mononuclear cells of such cases showed production of hCF by $CD4^+$ T cells but the number of hCF-producing cells did not correlate with the severity of the illness. The number of such cells may not indicate the amount of hCF produced as it may depend upon factors that trigger or down regulate the hCF-producing cells. What regulates the over production of hCF in some patients is not known; is it the dose of the virus or some humoral factor/cytokine or the genetic predisposition of the host? We know that production of mCF is much greater in inbred strains of mice than in the out-breds (unpublished results). Furthermore, it is not known whether hCF-producing cells are Th1 or Th2 or yet another subset of $CD4^+$ T cells. It appears possible that hCF-producing cells are either Th2-type or are regulated by Th2-cytokines. [31,32]. The production of mCF/hCF precedes the clinical illness in mice and man [30–33,36]. The DHF-like pathological lesions produced by mCF/hCF can be prevented by pretreatment of mice with the specific antibodies [38]. This has led to active vaccination of mice using mCF as antigen. The immunized mice were protected against subsequent challenge with mCF. Challenge of such mice with a lethal intracerebral dose of DV resulted in absence of clinical symptoms of the disease. These studies suggest a vaccine strategy should be directed against the primary cause of the disease (the cytokine) rather than the infective agent. At present an effective vaccine against dengue is not yet available [39]. Similar strategies are being successfully used in several diseases using anti-TNF-α antibody therapy [40]. While the level of TNF-α is increased in a variety of conditions, mCF/hCF have the advantage of being present only in dengue. We have shown that mCF is not produced during infection with an antigenically related Japanese encephalitis virus an unrelated polio virus, coxsackie or ECHO viruses and a number of bacteria and mitogens (reviewed in [5,30]).

6. Proposed mechanism of pathogenesis in DHF

With the available data, we would like to propose a mechanism that may explain the pathogenesis of DHF (Fig. 1). DV replicates in macrophages and induces quickly the CD4+ T cells to produce a unique cytokine, hCF. hCF induces macrophages to produce free radicals, nitrite, reactive oxygen and peroxynitrite [41,42]. 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 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 patients. The vascular permeability is increased due to the combined effect of histamine, free radicals, proinflammatory cytokines and the products of the complement pathway etc. Thus the key player appears to be hCF, but what regulates its activity is not known.

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