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MINIREVIEW

Dengue in infants: an overview

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Abstract

Dengue virus (DV) infection causes either a benign syndrome, dengue fever, or a severe syndrome, dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS), that is characterized by systemic capillary leakage, thrombocytopaenia and hypovolaemic shock. DHF/DSS occur mainly due to secondary infection by a heterotype DV infection in children and adults but in infants even primary infection by DV causes DHF/DSS. Clinical manifestations of DHF/DSS are more significantly associated with death in infants compared with older children. Vertical transmission of DV and anti-DV IgG has been well reported and is responsible for the pathogenesis of DV disease and its manifestations in infants. The complex pathogenesis of DHF/DSS during primary dengue in infants, with multiple age-related confounding factors, offers unique challenges to investigators. Dengue in infants is not often studied in detail due to practical limitations, but looking at the magnitude of DHF/DSS in infants and the unique opportunities this model provides, there is a need to focus on this problem. This paper reviews existing knowledge on this aspect of DV infection and the challenges it provides.

Introduction

Dengue viruses (DV) occur as four antigenically related but distinct serotypes transmitted to humans by *Aedes aegypti* mosquitoes. These viruses generally cause either a benign syndrome, dengue fever (DF), or a severe capillary leakage syndrome, dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) (Basu & Chaturvedi, 2008). DV affects humans of all age groups, worldwide. In some parts of the world it is mainly a paediatric public health problem, whereas in others such as Thailand dengue in infancy is also a serious medical concern (Pancharoen & Thisyakorn, 2001). Infant DHF/DSS was first reported in 1970 (Halstead *et al.*, 1970). DHF/DSS occurs in infants < 1 year of age born to dengue-immune mothers and in children 1 year and older who are immune to one serotype of DV and are experiencing infection with a second serotype. In Asia, dengue disease predominantly affects children, in whom it is among the 10 leading causes of hospitalization and death (Halstead *et al.*, 2002). Infants represent 5% of children hospitalized with DHF in Thailand (Pancharoen & Thisyakorn, 2001), Vietnam (Chau *et al.*, 2008), Myanmar (Halstead *et al.*, 2002), Indonesia (Graham *et al.*, 1999), Sri Lanka (Lucas & Mendis, 2001) and India (Kabilan *et al.*, 2003). Approximately one-quarter of these cases are DSS (Halstead *et al.*, 2002; Kalayanarooj & Nimmannitya, 2003). In the Americas, the incidence of DHF is highest among infants in Venezuela (San Martín *et al.*, 2010).

Prevalence of dengue disease in infants

Age-related differences have been identified in the prevalence of dengue diseases and their specific clinical manifestations. Infants are at high risk for DHF/DSS. In South East Asia, the age-specific incidence of infant DHF was 0.5 per 1000 persons over the age of 3–8 months, and it disappeared by age 9 months (Capeding *et al.*, 2010). Data from the first published study to estimate age-specific dengue hospitalization rates for the Bangkok metropolitan area show that in 1964, the hospitalization rate for infants with DHF/DSS was twice that of children in Bangkok during the same year (Halstead *et al.*, 1969). In a later report, infant DHF/DSS constituted 4.6–5.0% in Thai, Vietnamese, Myanmar and Indonesian infants and children hospitalized with DHF during 1995–1998 (Halstead *et al.*, 2002). In Thailand, dengue in infancy (≤ 2 years of age) is a serious medical problem, constituting 7.7% and 2.9% (rates observed in two different hospitals) of DV infections (Pancharoen &

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Thisyakorn, 2001). However, the death rate is low due to early diagnosis and prompt treatment (Witayathawornwong, 2001). Dengue in infancy has also been reported from Sri Lanka and India (Aggarwal *et al*., 1998; Lucas & Mendis, 2001). Dengue in infancy constituted 20% of total DV infections in an outbreak in Chennai, India. The major burden of DV disease lies in infants and children 5–9 years of age (Kabilan *et al*., 2003). Countries with a shorter or nonendemic history of DV circulation also report cases, principally in the adolescent and adult population. In countries where disease is endemic the distribution of the burden of disease is similar to that of DHF/DSS and/or severe clinical manifestations in both infants and children (da Cunha *et al*., 1995; Rahman *et al*., 2002). This difference may be due to variations in circulating DV serotype and strain or to changes in susceptibility to infection or enhanced disease due to immune status (Vaughn *et al*., 2000).

Manifestations of dengue disease in infants

Dengue is a well-documented public-health burden in many developing countries. Any of the four serotypes of DV can cause a spectrum of outcomes in humans, ranging from asymptomatic infection to mild and clinically significant severe disease (DHF/DSS), which is characterized by systemic capillary leakage, thrombocytopaenia and hypovolaemic shock (Table 1). The burden of severe illness lies predominantly in infants 4–9 months of age (Halstead *et al*., 2002). Severe clinical manifestations of DHF/DSS and DSS alone are more prevalent in infants, followed by children and then adults (Hammond *et al*., 2005). Manifestations such as convulsions and hepatic dysfunction more commonly affect infants than older children, and the fatality rate is four times higher in infants (Kalayanarooj & Nimmannitya, 2003). Only the frequency of internal haemorrhage increases with age, affecting one in seven adults (Hammond *et al*., 2005). The risk of presenting with signs of DHF/DSS by year of age was calculated and it was seen that infants and children aged 5–7 years are at significant risk for severe dengue, whereas in adults and adolescents 14 years and older, the risk is significantly less (odds ratio < 0.5) (Hammond *et al*., 2005). Haematocrit of $< 30\%$ is seen at a higher frequency among infants (infants $< 35\%$; older children $< 19.5\%$). Mean platelet count for infants ($56\,900\,mm^{-3}$) is significantly less than that for older children, and nearly 51.1% of infants have platelet counts of $< 80\,000\,mm^{-3}$ (Kabilan *et al*., 2003). Total white blood cell count and total lymphocyte count is significantly higher in infants than in children. Coryza, nausea/vomiting, rash, petechiae and other haemorrhagic manifestations are significantly higher in infants and younger children than in older children (Witayathawornwong, 2005). High-grade fever, hepatomegaly (Soundravally *et al*., 2010), rashes, oedema of the lower extremities, retro-orbital puffiness, vomiting and convulsions are commoner in infants than in older children (Kabilan *et al*., 2003). Infants with DHF/DSS present with some bleeding (87.5% of cases), cyanosis and ascites (37.5%) and shock (25%), as well as hepatomegaly (Martínez *et al*., 1993).

Table 1. Findings more often seen in infants with DV infection than in children and adults with DV infection

Findings	Reference(s)
High death rate	Kalayanarooj & Nimmannitya (2003)
DHF/DSS	Hammond *et al*. (2005), Kabilan *et al*. (2003), Martínez *et al*. (1993), Witayathawornwong (2005)
Thrombocytopaenia	Poli *et al*. (1991), Kabilan *et al*. (2003), Witayathawornwong (2005)
Raised total leucocyte count	Hammond *et al*. (2005), Kabilan *et al*. (2003), Witayathawornwong (2005)
Lymphocytosis	Hammond *et al*. (2005), Kabilan *et al*. (2003), Witayathawornwong (2005)
Severe clinical manifestations (shock, plasma leakage, internal haemorrhage)	Hammond *et al*. (2005), Witayathawornwong (2005)
Cynosis	Martínez *et al*. (1993)
Convulsion	Kabilan *et al*. (2003)
Hepatic dysfunction and hepatomegaly	Kabilan *et al*. (2003), Soundravally *et al*. (2010)
Splenomegaly	Hammond *et al*. (2005), Kabilan *et al*. (2003)
Ascites	Martínez *et al*. (1993)
Nausea/vomiting	Kabilan *et al*. (2003)
Rash/petechiae	Kabilan *et al*. (2003), Witayathawornwong (2005)
High-grade fever	Kabilan *et al*. (2003), Martínez *et al*. (1993), Witayathawornwong (2005)
Oedema of lower limbs	Kabilan *et al*. (2003), Martínez *et al*. (1993)
Retrobulbar puffiness	Kabilan *et al*. (2003), Martínez *et al*. (1993)
Coryza	Witayathawornwong (2005)

Vertical transmission of DV

Transplacental transmission of DV has been reported either by virus isolation (Thaithumyanon *et al*., 1994) or by detection of viral nucleic acid (Phongsamart *et al*., 2008). Some researchers have demonstrated the presence of anti-DV IgM in sera of a newborn as documentary proof of vertical transmission of the virus (Fernández *et al*., 1994). DV infection during pregnancy poses significant risk to both mother and child, while maternal antibodies are not harmful to the foetus or newborn (Table 2). A recent report from Malaysia (Chin *et al*., 2008) reported vertical transmission of acute dengue. A subject delivered a normal term baby girl

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Table 2. Foetal risks and dengue disease in pregnancy

Risks	References
Associated risks	
DHF/DSS	Poli *et al*. (1991), Chye *et al*. (1997), Chong & Lin (1989)
Stillbirth/death	Poli *et al*. (1991), Chye *et al*. (1997), Carles *et al*. (1999)
Thrombocytopaenia	Poli *et al*. (1991), Chin *et al*. (2008), Chye *et al*. (1997)
Low-grade fever	Poli *et al*. (1991), Chin *et al*. (2008), Chye *et al*. (1997)
Hepatomegaly	Poli *et al*. (1991), Chye *et al*. (1997)
Splenomegaly	Kabilan *et al*. (2003)
Coagulopathy	Poli *et al*. (1991), Chye *et al*. (1997)
Bleeding disorder	Poli *et al*. (1991)
Respiratory distress	Poli *et al*. (1991)
Vasomotor dysfunction	Poli *et al*. (1991)
Sepsis-like illness	Restrepo *et al*. (2003), Phongsamart *et al*. (2008)
Low birth weight	Restrepo *et al*. (2003)
Premature birth	Restrepo *et al*. (2003)
Foetal distress	Restrepo *et al*. (2003)
Nonassociated risks	
Peripartum haemorrhage	Chin *et al*. (2008)
Teratogenic effect	Chong & Lin (1989), Carles *et al*. (1999)
Abortion	Chong & Lin (1989), Carles *et al*. (1999)
Intrauterine growth retardation	Chong & Lin (1989), Carles *et al*. (1999)
No long-term sequelae	Phongsamart *et al*. (2008)

by spontaneous vaginal delivery and recovered uneventfully without peripartum haemorrhage despite the presence of thrombocytopaenia. The baby girl developed low-grade fever on day 4 and except for the transient thrombocytopaenia, recovered uneventfully following 3 days of mild illness (Chin *et al*., 2008). Presentation in infants can be mild, i.e. mild fever subsiding after 1 week uneventfully to hepatomegaly, thrombocytopenia and coagulopathy, or can involve bleeding disorder, respiratory distress, DHF/DSS and death. Clinical features of neonatal DV infections show that fever and vasomotor problems (blotches) are the dominating traits followed by hepatomegaly, thrombopaenia and DHF. In one study, two mothers had acute dengue, 4 and 8 days before the births of their infants; one mother had worsening of her proteinuric pregnancy-induced hypertension, liver dysfunction and coagulopathy and required multiple transfusions of whole blood, platelets and fresh frozen plasma. Her male infant was ill at birth, developed respiratory distress and a large uncontrollable left intracerebral haemorrhage, and died of multiorgan failure on day 6 of life. DV type 2 was isolated from the infant's blood, and IgM antibody specific to DV was detected in the mother's blood. The second mother had a milder clinical course; she gave birth to a female infant who was thrombocytopaenic at birth and had an uneventful hospitalization. DV type 2 was recovered from the mother's blood, and IgM antibody specific to DV was detected in the infant's blood. This report highlights not only the apparently rare occurrence of vertical transmission of DV in humans but also the potential risk of death for infected neonates (Chye *et al*., 1997). Passive transfer of maternal dengue antibodies to the foetuses influenced the occurrence of severe development of the disease (Martínez *et al*., 1993). Antibodies to DV in the dengue-infected mother can cross the placenta, which can cause newborn infants to develop DHF/DSS easily when they are primarily infected with DV. Anti-dengue activity was found in the lipid component of human milk and colostrum. This suggests that breast feeding will protect the infant from the DV in the endemic area of dengue infection (Chong & Lin, 1989).

Reports suggest that DV does not cause teratogenicity, abortion or intrauterine growth retardation of a foetus during pregnancy (Chong & Lin, 1989), whereas it is reported that DV may be responsible for foetal death (Carles *et al*., 1999), low birth weight, greater frequency of premature birth and increased foetal distress (Restrepo *et al*., 2003). Nine women infected with DF in early pregnancy received amniocentesis. Chromosome analysis revealed that all were normal, and the level of α-fetoprotein in amniotic fluids and maternal sera were within the normal range (Chong & Lin, 1989). In endemic areas, dengue infection can cause an acute febrile illness in pregnant women and sepsis-like illness in neonates. Vertical infection did not result in long-term sequelae (Phongsamart *et al*., 2008).

Antibody response during DV infection

DV genome is a single-stranded positive sense RNA which is translated as a single polyprotein that is cleaved by proteases of viral and host origin, to yield 10 viral proteins including the C and M proteins, the E glycoprotein, and seven nonstructural proteins (NS1, NS2A/B, NS3, NS4A/B, NS5). The antibody and T-cell responses to individual viral proteins are variable. Anti-E antibodies are the main response against DV that inhibit viral binding to cells, neutralize viral infectivity *in vitro*, protect mice from DV challenge on passive transfer and show a variable degree of cross-reactivity among the DV serotypes. Antibodies against NS1 can trigger complement-mediated lysis of DV-infected cells *in vitro* and protect mice from DV challenge.

Antibody-dependent enhancement (ADE) of DV infection

Antibodies produced by DV infections can be divided into (1) type-specific neutralizing antibodies, (2) cross-reactive non-neutralizing antibodies and (3) cross-neutralizing

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antibodies among the four DVs (Kurane & Takasaki, 2001; Stephenson, 2005). The cross-reactive, non-neutralizing antibodies remaining from earlier heterogeneous DV infection enhance the cellular uptake of DV and enhance the infection. They are also called enhancing antibody and cause ADE of infection (reviewed by Chaturvedi *et al.*, 2005).

DHF occurs almost exclusively in two settings: (1) children and adults who become infected with a second DV serotype after an initial 'primary' DV infection with a different serotype, and (2) infants with primary DV infections whose mothers have some DV immunity. The ADE model suggests that in individuals who develop DHF, although there are some antibodies against DV in their blood, these antibodies do not neutralize the virus, but rather they bind to it and enhance its uptake by certain Fc receptor-bearing monocytes/macrophages, thus increasing viral burden that results in more severe disease (reviewed by Halstead, 2003, 2009).

Secondary infections cause 40 times more DHF cases than primary infections. Neutralizing antibodies can reduce viraemia levels, whereas cross-reactive non-neutralizing (enhancing) antibodies may increase them. Individuals once infected with one serotype of DV are usually protected from subsequent infection with the same serotype (homotypic infection) (Halstead, 2003). Cross-protection against infections with different serotypes (heterotypic infection) is only shown in the short term following infection. A report shows that some individuals who had neutralizing antibodies against DV2 developed symptoms upon infection with DV2, suggesting that neutralizing antibodies do not always work for protection from the subsequent homologous infection (Endy *et al.*, 2004). Neutralizing antibody is important for protection against DV infection, as passive transfer of polyclonal and monoclonal (MAbs) neutralizing antibodies can confer protection from lethal challenge in a murine model (Chaturvedi *et al.*, 1977, 1978).

One study reported that enhancing antibodies do not correlate with viraemia level (Laoprasopwattana *et al.*, 2005). It has been demonstrated that all MAbs showing enhancing activities also show neutralizing activities. Addition of commercial rabbit complement or fresh human sera into the ADE assay systems abolishes the enhancing activities of all MAbs (Yamanaka *et al.*, 2008). The complement component C1q restricts ADE by antiflavivirus antibodies in an IgG subclass-specific manner in cell culture and mice. IgG subclasses that bind C1q avidly induce minimal ADE in the presence of C1q, whereas subclasses that bind C1q weakly enhance infection (Mehlhop *et al.*, 2007). The site of attachment of antibody to DV may be a determinant of ADE. Humanized MAbs directed at the fusion loop near the junction of domain I and II of the DV2 envelope protein enhance DV4 viraemia in rhesus monkeys (Goncalvez *et al.*, 2004, 2007), while the domain III antibodies mediate neutralization and protection functions (Pierson *et al.*, 2008).

DV infection of THP-1 cells via ADE suppresses nitric oxide radicals, by disrupting the transcription of the iNOS gene transcription factor, IRF-1, and blocking the activation of STAT-1. It also suppresses the transcription and translation of interleukin (IL)-12, interferon (IFN)-γ and tumour necrosis factor (TNF)-α, while the expression and synthesis of the anti-inflammatory cytokines IL-6 and IL-10 are enhanced. Thus, besides facilitating the entry process, DV infection via ADE also alters innate and adaptive intracellular antiviral mechanisms, resulting in unrestricted DV replication in THP-1 cells (Chareonsirisuthigul *et al.*, 2007). This is similar to the decreased nitric oxide and increased IL-10 blood levels seen in DHF patients (Ubol *et al.*, 2008; Chaturvedi & Nagar, 2009). Furthermore, DV infection of the FcR-bearing mast cell/basophil KU812 cell line through ADE results in a massive induction of apoptosis (Brown *et al.*, 2009). The abundance of IFN type I gene transcripts in peripheral blood mononuclear cells from Vietnamese DSS patients was lower than in those from DHF patients without shock (Simmons *et al.*, 2007b). The role of ADE in DHF/DSS is summarized in Fig. 1.

Maternally derived anti-dengue IgG in infants

The role of maternal dengue-specific antibodies in the development of DHF/DSS caused by DV2 in infants was reported by Kliks *et al.* (1988). Evidence of DV infection and very low geometric mean titres against all dengue serotypes in children at 6 months of age and beyond was demonstrated. DV infection rates increase from 12 months of age

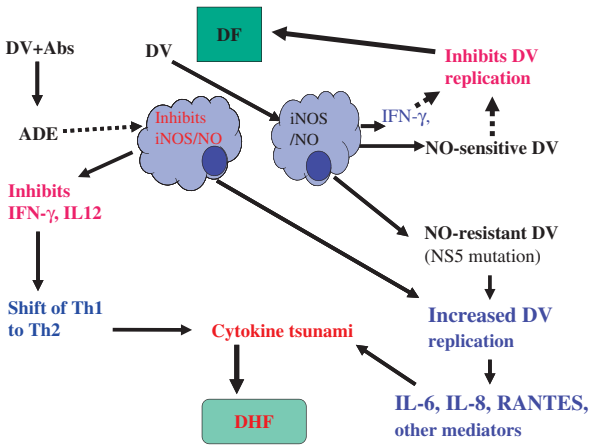

Fig. 1. Role of ADE during DV infection of dendritic cells/macrophages that may determine whether it will lead to DF or DHF (based on Chaturvedi & Nagar, 2009).

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onward. Maternal IgG antibodies are known to cross the placental barrier, providing protection to newborns against infections. Antibody titres in newborn are proportional to maternal antibody level (Perret *et al.*, 2005). The first report of dengue antibodies crossing the placental barrier in humans was that of Ventura *et al.* (1975), but these antobodies were not suspected to sensitize infants to DHF/DSS. Cord blood leucocytes from neonates with maternal dengue antibody supported DV2 replication *in vitro*; those from neonates without maternal antibody did not. Marchette *et al.* (1979) reported that cord blood of infants born to dengue-immune mothers contained a potent enhancing factor, which gradually decayed with age and which was absent from neonates born to nonimmune mothers. This supported the hypothesis that severe primary DHF with shock seen in Bangkok infants is related to maternal immune status (Marchette *et al.*, 1979).

ADE and DHF in infants

DHF in children and adults is typically associated with secondary infection caused by a DV serotype distinct from that present when an individual is first exposed to DV (Graham *et al.*, 1999). In contrast, DHF occurs in primary DV infections in infants born to dengue-immune mothers (Simmons *et al.*, 2007a). The bimodal age distribution of DHF was one of the earliest clues that alerted researchers to the immune enhancement theory. The age peaks occur at 7 months and 3–5 years. Children with secondary infections and DHF have higher initial plasma viral loads (Wang *et al.*, 2006).

Kliks and colleagues' investigation of DHF in infants found that maternal dengue 2 neutralization antibody titre and age of the infant were strongly correlated. The actual age at which DHF/DSS occur in infants correlates with the age at which maximum enhancing activity for dengue infection in mononuclear phagocytes is predicted. This critical time for the occurrence of DHF/DSS was observed to be approximately 2 months after the time calculated for maternal dengue-neutralizing antibodies to degrade below a protective level. In addition, sera of mothers of infants with DHF/DSS enhanced DV infection to a slightly greater degree than did sera from mothers of infants with pyrexia of unknown origin and toddlers with DHF/DSS. This showed that maternal dengue antibodies play a dual role by first protecting and later increasing the risk of development of DHF/DSS in infants who become infected by dengue 2 viruses (Kliks *et al.*, 1988). At birth, maternal antibodies protect infants from dengue infection. As IgG antibodies are catabolized, a period of risk to enhanced infection ensues, followed in turn by the loss of enhancing antibodies and a corresponding decline in risk for DHF/DSS (Kliks *et al.*, 1988, Fig. 2). Enhancement of infant infection by cord blood antibodies is also described in naturally infected kittens born to queens immune to feline infectious peritonitis virus (Horzinek & Osterhaus, 1978).

Maternal dengue antibody decay has been studied. A 1-year follow-up period reported that maternal haemagglutination inhibition antibodies against DV disappear by the age of 9 months in 99% of cases (Watanaveeradej *et al.*, 2003). In another study, 13% of 9-month-old infants still had lower titres of neutralizing antibody against at least one DV serotype. One infant had undifferentiated febrile illness due to DV2 infection 6 weeks after having been seen to have neutralizing antibodies against all four serotypes (Pengsaa *et al.*, 2003). In healthy Vietnamese infants, there was a strong temporal association between the Fc-dependent, DV infection-enhancing activity of neat plasma and the age-related epidemiology of severe dengue. A prospective, nested case-control study of primary DV3 infections during infancy has shown a full range of disease severity (Libraty *et al.*, 2009). Disease severity in infants with primary infections is associated with a robust immune response, possibly as a consequence of higher viral burdens *in vivo* (Chau *et al.*, 2008). Total IgG and DV-reactive IgG were higher in cord plasma than in the mother's plasma. Maternal DV-neutralizing and E protein-reactive IgG titres decline to below measurable levels in $>$ 90% of infants by 6 months of age, while DV-reactive IgG with whole DV virions persist until 12 months of age in 20% of infants. DV infection in infants is frequently subclinical but there is a window between 4 and 12 months of age where virion-binding but non-neutralizing IgG could facilitate ADE (Chau *et al.*, 2009). DV-infected cells release a high proportion of immature prM-containing virions that lack the ability to infect cells. However, they become highly infectious upon interaction with prM antibodies. During a secondary infection or primary infection of infants born to dengue-immune mothers, immature particles have the potential to be highly infectious and hence may contribute to the development of severe disease (Rodenhuis-Zybert *et al.*, 2010).

Cellular immunity and DV infection

The cellular immune response is a coordinated differentiation of heterogeneous populations of antigen-specific T cells (both CD4 and CD8) that effectively eradicate pathogens and provide protection against reinfection. Antigen-exposed T cells have been identified as three subsets: short-lived effector T cells, central memory T cells (Tcm) and effector memory T cells. The early delineation of these cells is regulated through specific transcription factors and cytokines; the latter are also responsible for the maintenance of Tcm cells. The heterogeneity of CD 4 T cells that play a role in DV infection is summarized in Fig. 3.

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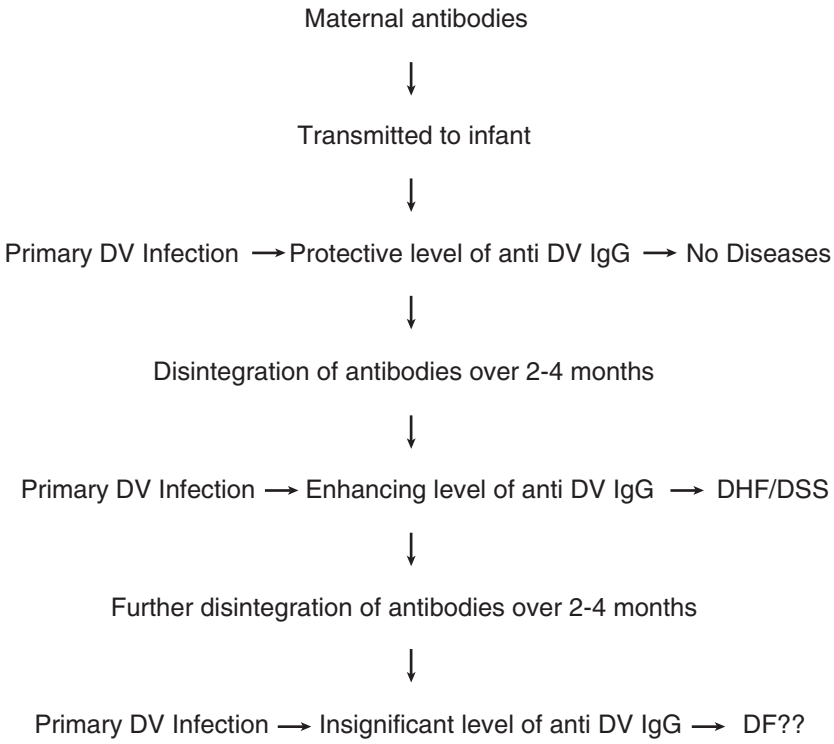

Fig. 2. Understanding the humoral immunopathogenesis of DHF/DSS during primary DV infection in infants.

T cells in infants

Little is known about the generation of the T-cell pool in healthy children. Normally, peripheral blood naive T-cell numbers are high in newborns and decline with increasing age, while memory T-cell numbers appear less dependent on age. It has been shown that during foetal life, mature T-cell responses can be elicited by viral and parasite infections (King *et al*., 2002; Marchant *et al*., 2003). T cells capable of producing IFN-γ increase progressively with age (Härtel *et al*., 2005). A significant age correlation has been observed in the expression/release of IFN-γ, IL-2, IL-4 and TNF-α. The mRNA levels of these cytokines increase from the neonatal period to infancy. The expression of IL-5 and IL-10 is usually not correlated. The expression of IL-12 in monocytes is also age-correlated (Härtel *et al*., 2005). Schönland *et al*. (2003) have suggested that increased T-cell division in young mice and children may be driven by high IL-7 levels, other growth factors and antigen exposure in the first year of life. The physiological immaturity of the immune system at birth has been attributed to the prevalence of suppressor factors during foetal life and antigenic naivety. The regulatory T cells (Treg) consist of a heterogeneous collection of cells that have the ability to suppress the activation and proliferation of other T cells and the immune response as a whole. Treg cells can inhibit protective antiviral responses and enhance disease progression. These cells can block T-cell activation, limiting viral replication as well as consequent immunopathology. Furthermore, inhibition of antiviral T-cell responses by Treg cells could lead to viral persistence and more rapid disease progression (reviewed by Chaturvedi *et al*., 2007). Treg cells are known to be present at higher frequency during human foetal ontogeny (Cupedo *et al*., 2005) and may be important in the context of perinatal infection.

Role of T cells in DV infection of infants

Cellular immune responses in infants with DV infection have not been well studied. Dengue immunopathogenesis has focused on the sequential DV infection phenomenon, suggesting that severe disease results from amplified cytokine release caused by dengue infections occurring in the presence of T-cell memory (Rothman & Ennis, 1999). This model cannot explain DHF/DSS during a first dengue infection (Fig. 4). Plasma levels of cytokines/chemokines are generally higher in infants with DSS. Antigen-driven inflammatory cytokine release is hypothesized to contribute to dengue capillary-leakage syndrome. Chemokine expression may be an early event in dengue and might play a role in immunopathogenesis. The concentrations of cytokines

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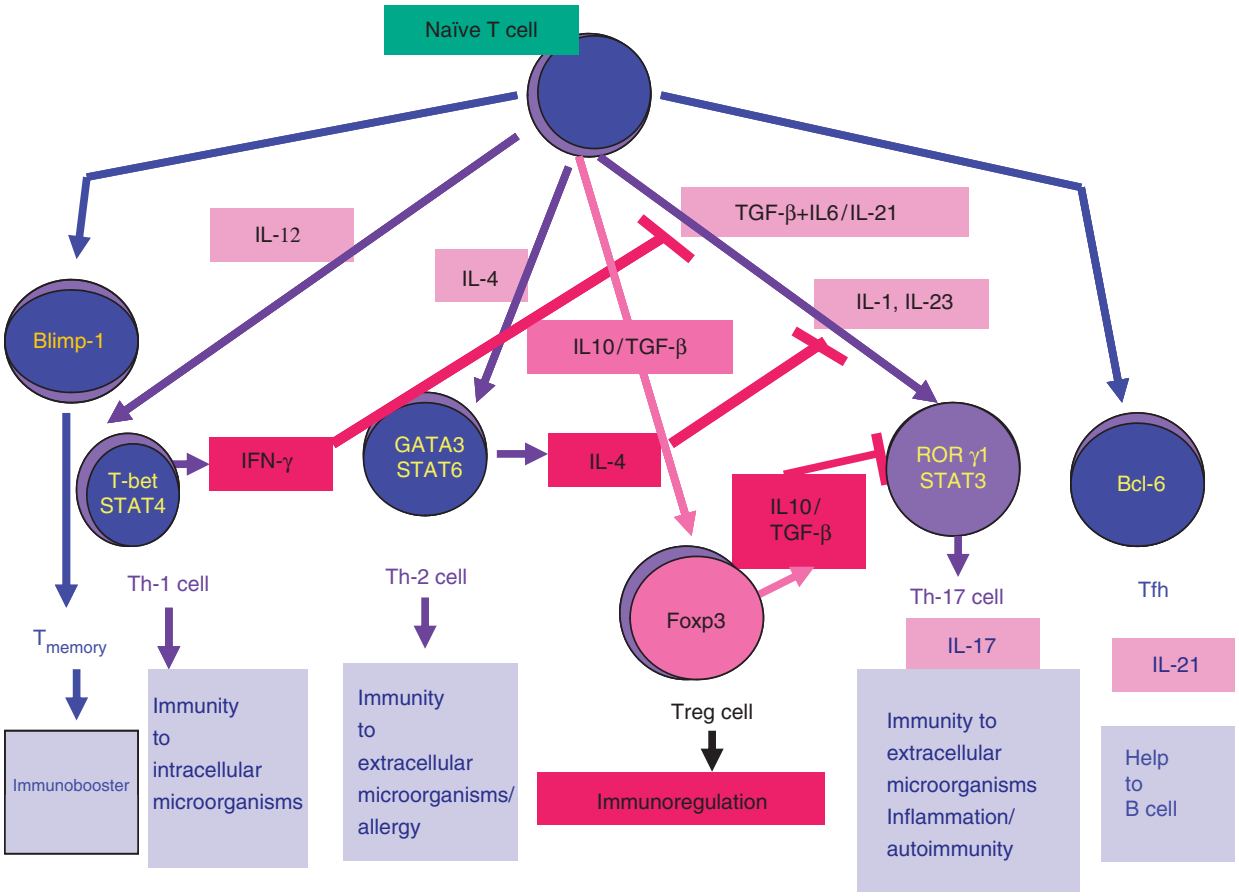

Fig. 3. Cytokines and transcription factors responsible for the generation of various subsets of CD4 T cells that may play roles in the pathogenesis of DHF.

(IL-1β, IL-6, IL-8, IL-10, IL-12p70 and TNF-α) and chemokines (IP-10, MCP-1, MIG and RANTES) in plasma samples from (except that of RANTES) infants with DSS are high. This is consistent with a relationship between the magnitude of immune inflammation and capillary leakage (Chau *et al.*, 2008). [Correction made on 23 April after online publication: IL-10 corrected to IP-10 in this paragraph.] Plasma levels of IP-10 correlate with viraemia, and RANTES and MIG correlate with hepatic dysfunction during acute primary dengue in infants. Plasma levels of other cytokines or chemokines in infants are not significantly correlated with viral loads, NS1 levels or other measures of disease severity including thrombocytopaenia, haematocrit and dengue disease grade. In infants, IP-10 is significantly correlated with plasma DV loads in the same sample. IP is expressed by hepatocytes in DV-infected mice and promotes infiltration of natural killer cells into hepatic tissue (Chen *et al.*, 2006). IP-10 and DV appear to compete for attachment to heparin sulphate on the cell surface, suggesting that IP-10 might have anti-DV activity beyond its immunological properties (Chen *et al.*, 2006). Plasma levels of RANTES and MIG are associated with liver transaminase levels, which may suggest a relationship between hepatic dysfunction and these chemokines, activities of which include the recruitment of lymphocytes to sites of infection. DHF in secondary infections is also associated with both significantly greater plasma concentrations of inflammatory cytokines (Chaturvedi *et al.*, 2000; Juffrie *et al.*, 2001) and increased frequencies of activated lymphocytes, compared with those in patients with milder disease (Mongkolsapaya *et al.*, 2003), presumably in response to higher viral burdens in DHF.

Cellular immune activation is associated with dengue severity during primary infection of infants as well as during secondary infection of older children (Juffrie *et al.*, 2001). The concept of antigen-driven immune activation in infants is consistent with observations in older children with secondary infection, in whom relatively high early viraemia levels and robust activation of lymphocytes and inflammatory cytokine responses have been found to be associated with disease of greater severity (Juffrie *et al.*, 2001). Infants with primary acute DHF show a significant positive association between dengue severity and markers of lymphocyte activation (Chau *et al.*, 2008). $NS3^{-}_{133-142}$-specific $CD8^{+}$ cells may play a role in the immunopathogenesis of

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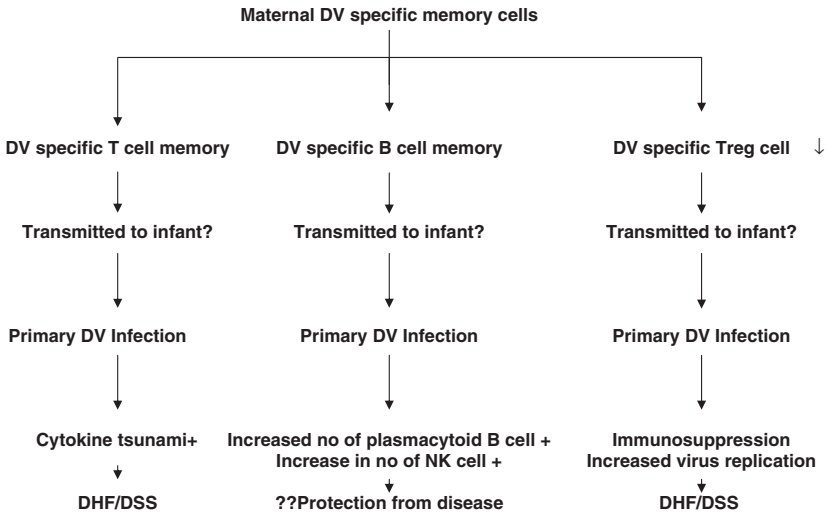

Fig. 4. Understanding the cellular immunopathogenesis of DHF/DSS during primary DV infection in infants. [Correction made on 23 April after online publication: Fig. 4 replaced with revised version.]

secondary infection, where responses can be expected to be earlier and dominated by robust serotype-cross-reactive responses, although these cells contribute little to the resolution of viraemia or immunopathogenesis in infants with primary dengue (Mongkolsapaya *et al.*, 2003).

Pathogenesis of DHF/DSS in infants

The findings described here indicate that maternally derived DV-reactive IgG determines the viral load and the pathogenesis in infants through ADE. Infection with DV through ADE inhibits induction of iNOS/nitric oxide and IFN-γ in dendritic cells/macrophages. In several studies, increased susceptibility of newborns to various infections has been attributed to a deficient secretion of IFN-γ (reviewed by Härtel *et al.*, 2005). Treg cells can suppress protective antiviral responses and accelerate disease progression. On the other hand, they can block T-cell activation, thus limiting viral replication and activation-associated immunopathology. It is interesting to note the findings of Simian immunodeficiency virus (SIV) infection in infant macaque monkeys that have a large number of highly suppressive Treg cells. These cells directly inhibit the CD4+ T-cell response to SIV, which is associated with a transient CD8+ T-cell response and limited antibody production (Hartigan-O'Connor *et al.*, 2007). Suppressor T cells are known to be present in both mice and humans infected with DV (Tandon *et al.*, 1979; Chaturvedi, 1984; Chaturvedi *et al.*, 2007; Lühn *et al.*, 2007). Similar mechanisms may work in infant patients with DHF/DSS, which may partly explain the vulnerability of human infants to DV infections. High viral burden *in vivo* may elicit consequent immune activation, which may be associated with disease severity and may contribute to dengue-related vasculopathy. In this situation, the burden of viral antigen and the magnitude of the immune response are important determinants of the clinical response. It has been suggested that high peak viraemia is necessary but not sufficient to cause plasma leakage; memory DV-specific T cells induced during a primary DV infection are reactivated by the heterologous viral serotype during a secondary infection to expand to high levels and produce a skewed cytokine profile (Rothman, 2009), resulting in a cytokine tsunami (Chaturvedi *et al.*, 2007; Chaturvedi & Nagar, 2009). During pregnancy, small number of various types of cells are transferred between the mother and the foetus via the placenta or breast-feeding (Zhou *et al.*, 2000). They include maternal erythrocytes, platelets, granulocytes and lymphocytes (Desai & Creger, 1963). Mold *et al.* (2008) have reported that substantial numbers of maternal cells cross the placenta to foetal lymph nodes, inducing the development of CD4+CD25highFoxP3+ Treg cells that suppress foetal antimaternal immunity and persist at least until early adulthood. Furthermore, Michaëlsson *et al.* (2006) have reported that foetal T cells are highly responsive to stimulation in the absence of Treg cells. It is worth investigating whether DV-specific memory cells also cross the placenta, and if so whether in sufficient numbers to initiate a cytokine tsunami. Several other explanations may be offered for faster disease progression in infants. This may be due to a lower intensity of adaptive T-cell responses resulting in unrestricted viral replication as the infants have lower frequencies of antigen-specific T helper (Th) cells with a Th2 cell bias. Furthermore, infant dendritic cells express lower levels of the major histocompatibility complex and adhesion molecules, their ability to produce IL-12 is lower and due to the limited expression of adaptor proteins and the limited activation of downstream signalling molecules the responses to Toll-like receptor ligation are reduced. This may compromise the priming of the T-cell response to DV in infants (Table 3).

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Table 3. Factors playing a role in DV pathogenesis in infants, children and adults

Factors	Infants (1–11 months)	Children (1–14 years)	Adults (> 15 years)	References
Severity of illness	64%	55%	36%	Hammond *et al*. (2005)
DHF/DSS	Primary infection (∼100%)	Secondary infection (50–95%)	Secondary infection (90%)	Hammond *et al*. (2005)
Antibody-dependent enhancement	Maternally derived IgG	Enhancing IgG, produced in primary infection	Enhancing IgG, produced in primary infection	Hammond *et al*. (2005)
Capacity to produce cytokines by T cells	Low	High	High	Hartigan-O'Connor *et al*. (2007)
CD4/CD8 ratio	High	Low	Low	Hartigan-O'Connor *et al*. (2007)
INF-γ and TNF-α production by naïve and memory T cells	Low	High	High	Hartigan-O'Connor *et al*. (2007)
IL-2 production by naïve T cells	Adequate	Adequate	Adequate	Hartigan-O'Connor *et al*. (2007)
Number of Treg cells in blood and tissues	High	Low	Low	Hartigan-O'Connor *et al*. (2007)
Immunosuppresion by Treg cells	High	Low	Low	Hartigan-O'Connor *et al*. (2007)
Antigen-specific T-cell response	Low	Adequate	Adequate	Rowe *et al*. (2001)
Th2 bias in response	High	No	No	Prescott *et al*. (1998)
Adaptive T-cell responses	Low	Adequate	Adequate	Hartigan-O'Connor *et al*. (2007)
Expression of MHC and adhesion molecules on dendritic cells	Low	Adequate	Adequate	Petty & Hunt (1998), Marchant *et al*. (2003)
IL-12 production by dendritic cells	Low	Adequate	Adequate	Goriely *et al*. (2001)

Conclusions and future perspectives

A large body of data suggests that DHF/DSS occurs during secondary dengue infections in adults and children and that the main inducers in this situation are ADE and memory T cells. The situation in infants is complex as DHF/DSS may accompany primary DV infection; the maternally derived specific IgG may be present but are memory T cells also present? It is not known how DV infection influences T-cell dynamics in infants. The complex pathogenesis of DHF/DSS during primary dengue in infants, with the confounding differences in age-related vascular physiology and immunological maturity, maternally derived memory cells and overwhelming Treg response, offers a unique challenge to investigators. The results in infants may not be directly applicable to older children/adults with secondary infections. Reduction of the viral burden by antiviral drugs or suppression of immune activation may help to improve clinical outcomes in infants (and also in children and adults) with dengue. Further studies will show whether interference with the regulatory control of CD4+ T cells can assist in control of dengue disease. Newer strategies are needed for the prevention and treatment of DHF/DSS. The magnitude of DHF/DSS in infants and the uniqueness of this model provide immense challenges for understanding pathogenesis. There is need to focus more attention on dengue disease in infants.

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