Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

ADVANCES IN VIRUS RESEARCH, VOL 59

MANIPULATION OF CELL SURFACE MACROMOLECULES BY FLAVIVIRUSES

Robert Anderson

Department of Microbiology & Immunology,
Dalhousie University
Halifax, Nova Scotia, B3H 4H7 Canada

Cell surface macromolecules play a crucial role in the biology and pathobiology of flaviviruses, both as receptors for virus entry and as signaling molecules for cell–cell interactions in the processes of vascular permeability and inflammation. This review examines the cell tropism and pathogenesis of flaviviruses from the standpoint of cell surface molecules, which have been implicated as receptors in both virus–cell as well as cell–cell interactions. The emerging picture is one that encompasses extensive regulation and interplay among the invading virus, viral immune complexes, Fc receptors, major histocompatibility complex antigens, and adhesion molecules.

0065-3527/03 $35.00

I. Introduction

Flaviviruses comprise a rich and diverse family of agents that infect a variety of hosts and cause a wide spectrum of disease. Three disease types are recognized for flaviviruses, namely encephalitis, hemorrhagic fever, and fever–arthralgia–rash. Disease distinctions are not absolute, and overlapping pathologies among various flavivirus members are often observed. The ability of flaviviruses to cause such divergent clinical syndromes, associated with virus replication in a number of different organs, has profound implications for the types of cell surface molecules the virus recognizes as receptors. Mutational analyses of the flaviviral E protein have demonstrated a striking ability of flaviviruses to adapt to different cells and receptors. Given the considerable homologies among them, flaviviruses show a remarkable capacity to cause vastly different diseases with a minimum of alterations in the E protein.

The cell surface molecules, which act as receptors for flaviviruses, are only starting to be identified. In addition to providing the molecules involved in virus attachment and penetration, the host cell erects a battery of surface structures that mediate communication with other cells and trigger host defense and pathological processes. Many of these are modulated by flavivirus infection and contribute to the overall picture of pathogenesis.

II. The Flavivirus Receptor Binding Protein

The flavivirus E protein is a multifunctional protein involved in cell receptor binding (Anderson *et al.*, 1992; Chen *et al.*, 1996; He *et al.*, 1995) and virus entry via fusion with a host cell membrane (Rice, 1996). Some of the functional activities of the E protein, notably membrane fusion, are regulated by interaction with a second viral protein, prM. It is believed that the association of prM with E stabilizes certain pH-sensitive epitopes on the E protein, thereby preventing the conformational changes that normally occur at acidic pH and activate the fusogenic activity of the E protein (Allison *et al.*, 1995; Guirakhoo *et al.*, 1992; Heinz *et al.*, 1994). In addition to its normal role in flavivirus assembly, the prM protein has also been included in novel recombinant formulations in which it is generally coexpressed with the E protein; the resultant E/prM complexes have been shown to be immunogenic and protective as vaccines against challenge with several flaviviruses, including Japanese encephalitis virus (Mason *et al.*,

1991), yellow fever virus (Pincus *et al*., 1992), dengue virus (Fonseca *et al*., 1994), and tick-borne encephalitis (TBE) virus (Heinz *et al*., 1995).

In TBE virus, the majority of extracellular virus is largely free of prM protein due to a late intracellular processing event that generates a carboxy-terminal fragment designated M and which together with the E and C proteins are believed to constitute the protein components of the mature virus particle (Heinz *et al*., 1994). Cleavage of prM to M enhances low pH–dependent virus–cell fusion (Guirakhoo *et al*., 1991) and infectivity (Guirakhoo *et al*., 1992; Heinz *et al*., 1994; Randolph *et al*., 1990; Shapiro *et al*., 1972; Wengler, 1989). Dengue virions containing prM are still infectious (Randolph *et al*., 1990) and bind to permissive cells in a manner that can be blocked using E-specific antibodies (He *et al*., 1995; Wang *et al*., 1999). Virus particles containing mainly E and prM also show antibody-enhanced binding to Fc receptor-bearing K562 cells as well as to platelets (Wang *et al*., 1995). Thus, in addition to being requisite precursors to mature virus particles, virus particles containing prM possess many properties associated with mature virus particles.

Flaviviruses appear to gain entry to the cell by the endocytic pathway (Rice, 1996). At low pH, the E protein undergoes a conformational change (Allison *et al*., 1995) involving dissociation of the E dimer (Stiasny *et al*., 1996), thereby exposing a hidden fusion peptide, followed by reorganization of E into a trimer (Allison *et al*., 1995), in which the fusion peptide is brought close to the membrane-anchoring carboxy terminus (Ferlenghi *et al*., 2001). Remarkably similar structural features and conformational rearrangements have been noted between the flavivirus E protein and the alphavirus E1 (Heinz and Allison, 2001; Lescar *et al*., 2001; Pletnev *et al*., 2001; Strauss and Strauss, 2001), suggesting a common evolutionary origin for these two virion surface proteins.

Considerable homology exists among flaviviral E proteins, raising the possibility that different flaviviruses may have similar receptor-binding motifs. For example, many mosquito-borne flaviviruses contain an RGD sequence (e.g., residues 388–390 of the Murray Valley encephalitis virus E protein), which has been implicated in virulence (Lobigs *et al*., 1990) and receptor binding by analogy with integrin-binding motifs (Rey *et al*., 1995). Mutagenesis studies of the yellow fever virus (Van der Most *et al*., 1999) and Murray Valley encephalitis virus (Hurrelbrink and McMinn, 2001) RGD motifs, however, have cast doubt on the role of integrins in flavivirus attachment or entry.

Studies with TBE virus have identified important determinants for pathogenicity within the suspected receptor-binding site on the upper-lateral surface of domain III (Mandl *et al*., 2000). Acquisition of heparan sulfate-binding mutations by passaging TBE in cell culture has also implicated amino acids in this region in receptor binding (Mandl *et al*., 2001). The selection of virus mutants on the basis of weak binding to brain membranes has been used with several neurotropic flaviviruses (Holbrook *et al*., 2001; Ni and Barrett, 1998; Ni *et al*., 2000) and has identified a variety of mutations within domain III as well as other regions of E. For dengue virus, blocking of virus cell binding correlates more closely to virus neutralization for mAb 3H5 than for mAb 1B7 (Wang *et al*., 1999). This may suggest that mAb 3H5 neutralizes dengue virus predominantly by blocking virus–cell attachment, whereas mAb 1B7 neutralizes dengue virus largely by a postattachment mechanism. The mAb 3H5-binding site on the dengue viral E protein has been partly characterized (Hiramatsu *et al*., 1996; Megret *et al*., 1992; Trirawatanapong *et al*., 1992) and probably encompasses, at a minimum, residues 383–385 (Hiramatsu *et al*., 1996) within domain III. More recent data involving a larger number of monoclonal antibodies indicate that mAbs that interact with domain III are in fact the most effective blockers of virus–cell attachment (Crill and Roehrig, 2001). A putative heparan sulfate-binding site on the dengue-2 E protein is also located within this region (Chen *et al*., 1997), and comparative sequencing of dengue type 2 genomes has implicated amino acid 390 of the E protein as a major determinant of pathogenicity (Leitmeyer *et al*., 1999). The pH-dependent conformational "hinge" region (between domains I and II) of the E protein has also been implicated in virulence, receptor interaction, and/or membrane fusion (Hurrelbrink and McMinn, 2001; Lee *et al*., 1997; Monath *et al*., 2002). Further mutagenesis studies will undoubtedly help define the sites of the E protein involved in flavivirus–cell macromolecule recognition.

III. Cell Targets for Flaviviruses

A. Dendritic Cells

Transmission of flaviviruses to humans generally occurs via the bite of an infected mosquito or tick. In the case of dengue, inoculated virus is thought to first replicate in skin Langerhans (dendritic) cells (Palucka, 2000; Taweechaisupapong *et al*., 1996a, 1996b; Wu *et al*.,

2000). Dendritic cells have also been shown to be involved in the transport of intradermally inoculated West Nile virus to local draining lymph nodes, with a subsequent accumulation of leukocytes (Johnston *et al.*, 2000). It is likely that dendritic cells will prove to be efficient carriers of a wide number of flaviviruses from their cutaneous site of infection to lymphoid and possibly other tissues.

Given the importance of dendritic cells in initiating immune responses (Banchereau *et al.*, 2000), they probably play a pivotal role in stimulating host defense against invading flaviviruses. Dengue virus infection of immature myeloid dendritic cells has been shown to induce their maturation accompanied by the expression of major histocompatibility complex (MHC) class I and II antigens; the costimulatory molecules CD40, CD80, and CD86; and the dendritic cell marker CD83 (Libraty *et al.*, 2001). Such changes were seen in both dengue-infected and bystander cells, indicating that upregulation of cell surface molecules could be a consequence of virus infection as well as virus-induced cytokine expression. Similarly, Langerhans cells infected with West Nile virus, as well as an alphavirus, Semliki Forest virus, express increased cell surface MHC class II and appear to undergo maturation to a cell type similar to lymphoid dendritic cells (Johnston *et al.*, 1996). The efficient presentation of both MHC class I– and II–associated viral peptides on the surface of dendritic cells permits the generation of potent cytotoxic and helper T cell responses (see also Section V,A).

B. Monocytes and Macrophages

Monocytes and macrophages have long been recognized as major targets of flavivirus replication in the human host (Halstead, 1989; Halstead *et al.*, 1977; Scott *et al.*, 1980). They are also important host cells for the antibody-enhanced replication of certain flaviviruses (see Section IV,C). Because of their presence in the circulation, blood monocytes may be particularly important to the pathogenesis of hemorrhagic viruses, such as dengue. Because most of the pathological changes associated with dengue virus are hemostatic in nature, it is suspected that blood cells, particularly virus-infected blood monocytes, orchestrate many of these effects.

Dengue virus–infected human monocytes have been shown to be potent sources of vasoactive cytokines such as tumor necrosis factor (TNF)-α (Anderson *et al.*, 1997) and interleukin (IL)-1β (Chang and Shaio, 1994). Monocytes are also known producers of several other vasoactive mediators, including IL-6, platelet-activating factor (PAF), prostaglandins, thromboxanes, leukotrienes, and nitric oxide

(Bulger and Maier, 2000; Funk, 2001; Lefer, 1989; Maruo *et al*., 1992; Montrucchio *et al*., 2000; Szabo and Billiar, 1999), any of which could have powerful effects on endothelial cell physiology. A crucial aspect in understanding dengue pathogenesis will be the identification of additional vasoactive mediators, which trigger the key dysfunctional events in vascular integrity.

Various tissue macrophages are undoubtedly important in the pathogenesis of flaviviral diseases but have, to date, not received much attention. Skin mononuclear cells, pulmonary, splenic, and thymic macrophages and liver Kupffer cells have been recognized carriers of viral antigen (Halstead, 1989). In the liver, virus or viral antigen has been found in Kupffer cells and hepatocytes in infections with yellow fever (Monath *et al*., 1989) and dengue (Bhamarapravati *et al*., 1967; Hall *et al*., 1991; Halstead, 1989; Rosen and Khin, 1989). Destruction of Kupffer cells, possibly by apoptosis, has been reported in the liver of some patients with fatal dengue (Huerre *et al*., 2001). Primary cultures of Kupffer cells apparently undergo an abortive infection with dengue virus in which viral antigen but no progeny virus is produced (Marianneau *et al*., 1999).

C. Endothelial Cells

Many flaviviruses invade either visceral or central nervous system tissues following initial replication in dendritic cells, monocytes, or macrophages. Often this necessitates a transfer of virus across blood vessel endothelial layers.

For neurotropic flaviviruses, endothelial cells of the cerebral microvasculature constitute a barrier that must be overcome in order to gain access to the central nervous system. How this occurs remains uncertain. Transendothelial passage of virus may direct infection of cerebral microvascular endothelial cells, may transport across the endothelial layer, or both (Dropulic and Masters, 1990). Japanese encephalitis virus has been observed electron microscopically to traverse mouse cerebral endothelial cells by transcytosis (Liou and Hsu, 1998). Alternatively, virus may spread from blood vessels to the olfactory neuroepithelium and from there to olfactory neurons (McMinn *et al*., 1996; Monath *et al*., 1983).

Even normally nonneurotropic flaviviruses may occasionally invade the central nervous system under certain conditions. Modulation of the blood–brain barrier by anesthetics (Ben-Nathan *et al*., 2000) or lipopolysaccharide (Lustig *et al*., 1992) has been reported to facilitate neuroinvasion by a normally noninvasive strain of West Nile virus.

Flaviviruses may also trigger the production of soluble factors that perturb the integrity of the blood–brain barrier, leading to increased leakage of proteins and cells into the central nervous system (Chaturvedi *et al*., 1991). These studies indicate that even nonneurotropic flaviviruses may infect tissues of the central nervous system or otherwise affect the integrity of the blood–brain barrier under special circumstances.

Transendothelial migration of individual leukocytes (e.g., lymphocytes, monocytes, neutrophils, eosinophils) is regulated in a highly specific manner by the differential expression of selected adhesion molecules on endothelial cells (reviewed in Crockett, 1998; Lowell and Berton, 1999). Flaviviruses, including dengue (Anderson *et al*., 1997) and West Nile (Shen *et al*., 1997) viruses, activate endothelial cell adhesion molecule expression by either direct (virus-mediated) or indirect (cytokine-mediated) mechanisms (see Section V,C). In the presence of leukocyte-attracting chemokines, such virus-triggered activation of the vascular endothelium may contribute toward the migration of leukocytes into extravascular tissues. In addition to being a mechanism for virus dissemination, this process may also be a factor in phenomena such as leukopenia and particularly neutropenia (loss of circulating leukocytes, neutrophils) often observed in flavivirus, particularly dengue, infection (reviewed in Halstead, 1989). Due to the lack of suitable animal models for severe dengue disease, i.e., dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), there are difficulties in assessing the roles of such events, particularly the identification of adhesion molecules mediating the transendothelial migration of neutrophils using blocking antibodies against specific integrins, as has been performed for other disease states (Doerschuk *et al*., 1990; Gao *et al*., 1994; Issekutz and Issekutz, 1993; Laberge *et al*., 1995; Springer, 1995).

The hallmark feature of increased vascular permeability in hemorrhagic flavivirus (e.g., dengue) infection suggests that vascular endothelial cells may mediate the fluid leakage and hemorrhaging that occur in DHF/DSS. Endothelial cells line the inner surface of blood vessels and play essential roles in maintaining an antithrombogenic surface and regulating vascular permeability. Increased vascular permeability can arise from a variety of mediators associated with acute inflammation and shock (Bulger and Maier, 2000; Funk, 2001; Lefer, 1989; Michel, 1988; Montrucchio *et al*., 2000; Schnittler *et al*., 1990). It is thought that vascular permeability is largely controlled by changes in endothelial cell–cell contact, which result in gap formation, thus allowing for fluid exchange between blood and interstitial tissue

fluid (Michel, 1988). An electron microscopic study of endothelium from DHF biopsy samples revealed the occasional presence of gaps (Sahaphong *et al*., 1980), thus providing evidence that endothelial cell features may indeed be perturbed during DHF/DSS.

Although dengue virus infects endothelial cells *in vitro* (Andrews *et al*., 1978; Avirutnan *et al*., 1998; Killen and O'Sullivan, 1993), there is no evidence that endothelial cell infection occurs clinically, as neither virus particles nor viral antigen has been detected in the endothelium of tissue specimens (Halstead, 1988, 1989; Sahaphong *et al*., 1980), in contrast to that seen in cases of ebola (Zaki *et al*., 1999) or hantaan hemorrhagic fever (Gavrilovskaya *et al*., 1999; Wang *et al*., 1997). It is likely that dengue virus mediates endothelial cell activation via an indirect route, involving blood monocytes, which are a major cell target for dengue virus infection (Halstead *et al*., 1977b; Scott *et al*., 1980). A major candidate event in such a route is the activation of endothelial cell adhesion molecules by a factor(s) (particularly TNF-α) produced by dengue virus–infected blood monocytes (Anderson *et al*., 1997).

TNF is a key cytokine in a variety of normal and pathological immune responses, including immunoregulation, regulation of cell proliferation, cytotoxicity, and in the mediation of endotoxic shock (Fiers, 1991; Tartaglia and Goeddel, 1992; Tracey and Cerami, 1993; Vassalli, 1992). Monocyte-derived TNF-α appears to play a pivotal role in dengue-associated endothelial cell activation (Anderson *et al*., 1997) and may be an important effector in the manifestation of DHF/DSS. Support for the clinical significance of this observation comes from observations of elevated TNF levels in the sera of patients with severe dengue disease (Green *et al*., 1999b; Hober *et al*., 1993; Vitarana *et al*., 1991; Yadav *et al*., 1991). Taken together, current evidence indicates that dengue virus represents a rather unique group of viruses that target monocytes, thereby triggering the production of factors such as TNF-α, which in turn affect other cell targets, including endothelial cells. While the overall picture of endothelial cell dysfunction in DHF/DSS is obviously more complex than can be explained by any single factor, the role of TNF in dengue pathogenesis would seem to merit particular attention.

Current knowledge of endothelial cell responses observed in endotoxic shock may be instructive for the understanding of vascular leakage in DHF/DSS. Plasma leakage induced by endotoxin (lipopolysaccharide, LPS) from gram-negative bacteria encompasses a complex cascade of processes, including activation and functional alteration of endothelial cells. Major mediators of endothelial cell perturbation

in endotoxic shock are LPS itself, as well as cytokines such as TNF-α and IL-1β (Bevilacqua, 1993). These factors can modulate endothelial cell function to varying degrees by activating cytokine and vasoactive factor release (Rink and Kirchner, 1996; Shanley *et al*., 1995), upregulating adhesion molecule expression (Bevilacqua, 1993; Luscinskas *et al*., 1991; Moser *et al*., 1989; Smith *et al*., 1989), and mediating transendothelial migration of specific leukocytes (Issekutz *et al*., 1995; Luscinskas *et al*., 1991; Morzycki *et al*., 1990; Moser *et al*., 1989; Smith *et al*., 1989). Additional factors, particularly lipid mediators such as PAF, leukotrienes, thromboxanes, and prostaglandins, may contribute to further endothelial cell dysfunction, including vascular leakage (Bulger and Maier, 2000; Funk, 2001; Lefer, 1989; Montrucchio *et al*., 2000). While the involvement of these vasoactive mediators is recognized in endotoxic shock, more needs to be learned of their role in the vascular dysfunction that occurs in severe dengue disease.

D. Lymphocytes

Although lymphocytes are potently involved in the host response and immunopathology of flavivirus (especially dengue) diseases, their role as virus-permissive host cells is unclear. Dengue virus has been identified in circulating B cells from acutely ill dengue patients by immunocytochemistry and by recovery of infectious virus after passage in mosquitoes (King *et al*., 1999). *In vitro* studies showed that cells and cultured cell lines of both B and T cell derivation could be infected with dengue virus (Bielefeldt-Ohmann *et al*., 2001; Kurane *et al*., 1990; Marchette and Halstead, 1978; Mentor and Kurane, 1997; Sung *et al*., 1975; Takasaki *et al*., 2001; Theofilopoulos *et al*., 1976). Continued passage of dengue virus in lymphoblastoid (Raji) cells can give rise to dengue virus variants capable of replication in human lymphocytes (Brandt *et al*., 1979). Interestingly, lymphocytes do not appear to undergo antibody-enhanced dengue virus infection (Brandt *et al*., 1979; Kurane *et al*., 1990), even though B cells do have Fc receptors (Dijstelbloem *et al*., 2001; see Section IV,C).

E. Neural Cells

The initial stages of pathogenesis for neurotropic flaviviruses appear to be common for flaviviruses in general in that the virus progresses from the subcutaneous site of inoculation to lymph nodes, followed by viremia and replication in extraneural tissues. Invasion into the

central nervous system is marked by high virus titers in the brain and detectable virus or viral antigen in neurons (Albrecht, 1968). Cell destruction in tick-borne encephalitis may be less extensive than that seen in herpes simplex type 1 encephalitis (Studahl *et al.*, 2000), although this is variable and may involve considerable inflammation (Chu *et al.*, 1999; Matthews *et al.*, 2000; Suzuki *et al.*, 2000). Susceptible cell types include both neurons and glial cells (Chu *et al.*, 1999; Ramos *et al.*, 1998; Steele *et al.*, 2000).

F. Basophils/Mast Cells

As notorious producers of vasoactive mediators, mast cells have been a source of controversial speculation for years in dengue pathogenesis. Cells resembling degranulated mast cells have been reported in skin perivascular infiltrates from DHF/DSS cases (Bhamarapravati *et al.*, 1967). Dengue patients showed elevated levels of urinary histamine (a major granule product of mast cells), which correlated with disease severity (Tuchinda *et al.*, 1977), suggesting that mast cells may have a contributory role in the pathogenesis of dengue. Although antihistamine treatment does not resolve shock in severely dengue-diseased patients (Halstead, 1989), histamine is only one of several potent vasoactive factors produced by mast cells (Benyon *et al.*, 1991; Bradding *et al.*, 1993; Galli *et al.*, 1984; Grabbe *et al.*, 1994; Marshall and Bienenstock, 1994; Moller *et al.*, 1991, 1993, 1998; Nilsson *et al.*, 1995; Schwartz and Austen, 1984), some of which could cause vascular dysfunction in dengue infection. DHF/DSS patients have been reported to have elevated serum levels of IgE (Pavri *et al.*, 1979), which has been speculated to relate to IgE-triggered histamine release in the manifestation of shock (Pavri and Prasad, 1980).

Mast cells reside mainly in the tissues, often closely associated with blood vessels (Alving, 1991; Anton *et al.*, 1998; Pesci *et al.*, 1996; Pulimood *et al.*, 1998; Selye, 1966; Selye *et al.*, 1968). They are present in large numbers in the skin (Marshall *et al.*, 1987), where transmission of insect-borne flaviviruses occurs. Basophils, however, comprise about 1% of total circulating cells and would be accessible to virus in the blood. Dengue virus infects basophil/mast cell–like KU812 cells in an antibody-enhanced manner, coupled with the release of vasoactive cytokines, IL-1β and IL-6 (King *et al.*, 2000, 2002). This cell line, which can be differentiated easily toward either a basophil or mast cell phenotype (Saito *et al.*, 1995), may provide further insights into potential roles for basophils and mast cells in dengue disease.

Dengue patients show increased serum levels of anaphylatoxins C3a and C5a (Malasit, 1987), which can attract (Nilsson *et al.*, 1996) and activate (Kownatzki, 1982) mast cells. Among the expected mast cell secretion products would be vasoactive factors, including histamine, which has been detected in elevated amounts in the urine of dengue patients (Tuchinda *et al.*, 1977).

G. Platelets

Evidence for platelet involvement in dengue pathogenesis comes from at least two (probably related) sources. First, thrombocytopenia (loss of circulating platelets) is one of the most consistent clinical features of severe dengue infection (Halstead, 1989). Second, viral immune complexes have been detected on platelets from dengue patients (Boonpucknavig *et al.*, 1979; Phanichyakarn *et al.*, 1977a). Functional studies on platelets in dengue-diseased individuals have been sparse, but include a markedly reduced half-life (Mitrakul *et al.*, 1977), deficient ADP release (Mitrakul *et al.*, 1977), increased adhesiveness (Doury *et al.*, 1976), increased tagging by complement fragments (Malasit, 1987), and increased release of β-thromboglobulin and platelet factor 4 (Srichaikul *et al.*, 1989). There is also evidence for platelet activation in dengue patients (Doury *et al.*, 1976; Krishnamurti *et al.*, 2001; Srichaikul *et al.*, 1989). Although these results relate to a variety of platelet functions, they do indicate a general alteration in platelet physiology, which is consistent with platelet involvement and triggering of thrombocytopenia in dengue disease.

Dengue virus has been recovered from washed patient platelets (Scott *et al.*, 1978), and virus has been reported to bind to platelets in the absence of antibody as assayed using immunofluorescence and immunoperoxidase techniques (Funahara *et al.*, 1987). However, the levels of antibody-independent bound virus are very low compared to the levels of virus bound in the presence of dengue-specific antibodies (Wang *et al.*, 1995). As noted earlier, dengue immune complexes have been demonstrated on platelets from dengue patients (Boonpucknavig *et al.*, 1979; Phanichyakarn *et al.*, 1977a). Weiss and Halstead (1965) originally proposed the possibility that dengue virus interactions with platelets might be involved in the thrombocytopenia observed in severe dengue disease. The finding that dengue virus binding to platelets is dependent on a virus-specific antibody is consistent with epidemiological and experimental data linking preexisting host antibodies to an increased risk of DHF/DSS (reviewed in Halstead, 1990).

Several other viruses have been shown to bind directly to platelets (Bik *et al*., 1982; Danon *et al*., 1959; Forghani and Schmidt, 1983; Larke and Wheelock, 1970; Lee *et al*., 1993; Zucker-Franklin *et al*., 1990). Platelet association may stabilize or protect blood-borne viruses (Larke and Wheelock, 1970) and may function as a mechanism of hematogenous dissemination (Forghani and Schmidt, 1983). Virus binding to platelets has been suggested to be a contributing mechanism to thromobocytopenia arising from infections with vaccinia (Bik *et al*., 1982), chikungunya (Larke and Wheelock, 1970), and rubella (Bayer *et al*., 1965). Thrombocytopenia in these virus infections is generally much milder than that observed in severe dengue disease.

Levels of dengue virus in the blood can exceed 10^7 infectious units/ml (Gubler, 1988; Monath, 1994). Such high viremic titers are likely necessary to ensure infection and transmission of the obligate mosquito intermediary host (Monath, 1994). Assuming a reasonable particle:infectivity ratio of 100:1, virus particle titers in blood may rival normal platelet counts (3×10^8/ml). Such parity between numbers of virus particles and platelets suggests that antibody-enhanced binding of virus to platelets may have a profound effect on platelets. Circulating virus-immune complexes are detected in DHF/DSS, and levels of immune complexes have been correlated with severity of disease (Ruangjirachuporn *et al*., 1979) and some of these are platelet associated (Boonpucknavig *et al*., 1979; Phanichyakarn *et al*., 1977a). These observations suggest that sufficient binding of virus immune complexes to platelets may occur to tag the majority of circulating platelets. Such an event could lead to immune clearance by the reticuloendothelial system, thereby precipitating the thrombocytopenia frequently associated with severe dengue disease.

It is likely that molecules other than Fc receptors on the platelet surface may mediate antibody-enhanced binding of dengue virus (Wang *et al*., 1995). Drug-induced thrombocytopenias provide interesting examples in this regard. It is known that given the appropriate accessory ligand (i.e., drug), IgG can bind to platelets through either the Fc receptor or other surface proteins. A variety of clinical thrombocytopenias are known that involve an immune component in pathogenesis. Many of these reflect activities of host antibodies, which react with proteins on the surface of platelets. These antibodies may be autoimmune in nature (i.e., antibodies that bind to platelet surface molecules) or dependent on a third party ligand (drug or protein), which then induces binding of the antibody–ligand complex to either the platelet Fcγ receptor or to another surface protein. For example, a number of individuals are susceptible to drug-dependent

thrombocytopenia when administered drugs such as heparin or quinine/quinidine (Aster, 1989; Hackett *et al*., 1982). While heparin-dependent antibodies bind to the platelet Fcγ receptor (Adelman *et al*., 1989; Chong *et al*., 1989a, 1989b; Kelton *et al*., 1988), quinine/quinidine-dependent antibodies bind to platelet protein heterodimers GPIIb/IIIa and GPIa/IX (Berndt *et al*., 1985; Chong *et al*., 1983; Christie *et al*., 1987; Devine and Rosse, 1995). This latter category of immune-mediated thrombocytopenia may be relevant to the understanding of dengue-associated thrombocytopenia, as patient antibodies mediate dengue virus binding to platelets via a platelet surface protein other than the Fcγ receptor (Wang *et al*., 1995).

Communication between platelets and endothelial cells is a frequent intermediate step in certain events such as platelet adhesion, aggregation, and regulation of vascular permeability. How this occurs in dengue infection and what the effects are on endothelial cell function are unknown. Binding of viruses to platelets can have potentially profound immunological effects [e.g., the stimulation of TGF-β release by platelets bound by Epstein–Barr virus (Ahmad and Menezes, 1997)]. In light of reports of altered platelet function in dengue patients, discussed earlier, there is a tantalizing need to determine the immunological consequences of antibody-enhanced dengue virus binding to platelets in terms of platelet as well as endothelial cell physiological responses.

Many products of complement activation can also be deposited on platelets (Devine, 1992). In view of evidence for complement activation in severe dengue disease (Halstead, 1989; Malasit, 1987), binding of complement products might play a role in the immune destruction of platelets leading to thrombocytopenia. Platelets display surface receptors, e.g., C1q receptor (Peerschke and Ghebrehiwet, 1987, 1998), membrane cofactor protein (Seya *et al*., 1986), and decay-accelerating factor (Devine *et al*., 1987), for specific components of complement activation. In addition, the platelet surface can act as a substrate for the deposition of C3dg and C5b-9 (Devine, 1992). Fragments of C3 have been detected on the platelets of DHF/DSS patients (Malasit, 1987).

In addition to immune complex deposition on platelets, thrombocytopenia associated with DHF/DSS might also arise by the immune destruction of platelets through antiplatelet autoantibodies. Antiplatelet autoantibodies have been reported in the sera of dengue patients (Lin *et al*., 2001), although they have also been detected in patients recovering from a variety of viral infections (Imbach, 1994). Antiplatelet antibodies are strongly linked to the pathogenesis of immune-mediated thrombocytopenias, such as idiopathic thrombocytopenic purpura (Winkelstein and Kiss, 1997).

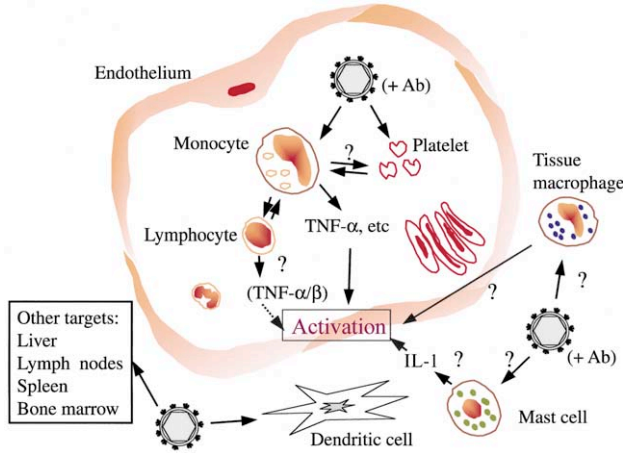

FIG 1. Model showing surface interactions of hemorrhagic flavivirus (dengue) with extra- and intravascular cell targets. Intravascularly, the presence of subneutralizing levels of antivirus antibodies stimulates virus attachment to platelets and infection of monocytes. This results in immune complex deposition on platelets and secretion of vasoactive factors from virus-infected monocytes. Among such vasoactive factors are cytokines, particularly TNF-α, which activates increased surface expression of adhesion molecules on endothelial cells. Extravascularly, virus infection of tissue macrophages, mast cells, and dendritic cells may result in the release of additional factors, which contribute to endothelial cell perturbation.

H. Cell Targets: An Overview

While this brief discussion of cell targets for flaviviruses is by no means complete, it highlights some of the major interactions as they relate to pathogenesis. Because pathogenesis is probably best understood for dengue, Fig. 1 illustrates the interactions of hemorrhagic flavivirus (e.g., dengue) with cell targets both within and outside the vascular system.

IV. CELL SURFACE MACROMOLECULES INVOLVED IN FLAVIVIRUS ATTACHMENT

A. Glycosaminoglycans

Glycosaminoglycans and proteoglycans (i.e., proteins bearing glycosaminoglycans) are important cell surface molecules involved in a variety of ligand recognition and cell signaling processes (Gallo, 2000). Because glycosaminoglycans are widely distributed on cells,

they are attractive candidates as virus receptors. Some degree of specificity (i.e., virus tropism) may arise from the compositional heterogeneity of glycosaminoglycans, as well as quantitative differences in the degree of expression on various cell types.

Flaviviruses seem to share, with a large number of virus families, the ability to bind glycosaminoglycans (Birkmann *et al*., 2001; Dechecchi *et al*., 2000, 2001; Duisit *et al*., 1999; Feldman *et al*., 1999, 2000; Giroglou *et al*., 2001; Goodfellow *et al*., 2001; Heil *et al*., 2001; Hsiao *et al*., 1999; Hulst *et al*., 2000, 2001; Lin *et al*., 2000; Liu and Thorp, 2002; Patel *et al*., 1993; Rue and Ryan, 2002; Shukla *et al*., 1999; Shukla and Spear, 2001). Glycosaminoglycans such as heparin and its structural analogues have been investigated for their ability to bind dengue virus and thereby to gain insights as to the structural requirements for dengue receptors. Potential glycosaminoglycan-binding motifs have been identified on the dengue viral E protein at two sites, the best characterized of which appears to be composed of amino acids 188, 284–295, and 305–310 and which may also play a role in virus–cell attachment (Chen *et al*., 1997). Heparin (minimum of 10 carbohydrates) and an uncharacterized highly sulfated heparin sulfate isolated from bovine liver were found to show the best binding to dengue E protein (Chen *et al*., 1997). Attachment of dengue virus to human hepatoma cells has also been reported to be inhibited by heparin (Hilgard and Stockert, 2000). A further study involving a panel of natural and synthetic polyanionic, sulfated compounds suggested that binding of the dengue E protein required a highly sulfated (and highly charged) oligosaccharide with a minimum size of 39Å and a high degree of structural flexibility (Marks *et al*., 2001).

The role of glycosaminoglycans in natural (i.e., nontissue culture-adapted) strains of flaviviruses needs to be studied further. It has long been recognized that dengue virus passaged in various host cell types can give rise to virus variants with altered cell specificity (Brandt *et al*., 1979; Halstead *et al*., 1984a, 1984b, 1984c). Passage-dependent mutations of the dengue virus E protein at a number of different amino acid residues have been documented (Lee *et al*., 1997). Following passage of TBE virus in cultured BHK-21 cells, virus mutants were selected that contained more positively charged amino acids in the putative receptor-binding region of the E protein, resulting in dependence on cell surface heparan sulfate (Mandl *et al*., 2001). Such mutants were diminished in their neurovirulence in mice as well as in their replication in primary chicken cells and plaque formation in porcine kidney cells (Mandl *et al*., 2001). A large number of other viruses have also been shown to undergo loss of virulence upon adaptation to cell culture

associated with heparan sulfate utilization (Bernard *et al*., 2000; Byrnes and Griffin, 2000; Klimstra *et al*., 1998, 1999; Lee and Lobigs, 2000; Neff *et al*., 1998; Sa-Carvalho *et al*., 1997).

B. CD14

CD14 and the Toll-like receptor (TLR) pattern recognition receptors are involved in the innate response to lipopolysaccharide and other microbial products (Diamond *et al*., 2000; Imler and Hoffmann, 2000). A role for CD14 and TLR4 has been found for respiratory syncytial virus (RSV) (Kurt-Jones *et al*., 2000), suggesting that these receptors may have a broader involvement in host response than previously thought. A possible role for CD14 in dengue infection has been postulated on the basis of inhibition of dengue virus infection of human monocytes with bacterial lipopolysaccharide (Chen *et al*., 1999). However, this has been disputed (Bielefeldt-Ohmann *et al*., 2001) and requires further investigation.

C. Fc Receptors

As indicated earlier, flaviviruses are capable of initiating infection of appropriate host cells through as yet largely unidentified primary receptors. In addition, a number of flaviviruses are capable of using subneutralizing levels of virus-specific antibodies to attach to and gain entry to cells bearing Fc and/or complement receptors (Cardosa *et al*., 1983; Halstead, 1982; Halstead and O'Rourke, 1977a; Schlesinger and Brandriss, 1981a) by a process known as antibody-dependent enhancement (ADE) of infection (Table I). ADE has been documented for dengue (Halstead *et al*., 1980), West Nile (Peiris and Porterfield, 1979), yellow fever (Schlesinger and Brandriss, 1981b), tick-borne encephalitis (Phillpotts *et al*., 1985) and Japanese encephalitis (Cecilia and Ghosh, 1988) viruses. Early work with dengue virus and monocytes differentiated between trypsin-sensitive and trypsin-resistant cell surface molecules as the putative receptors for antibody-independent and antibody-dependent infection, respectively (Daughaday *et al*., 1981).

To date, dengue virus appears to be the only flavivirus in which strong evidence exists for antibody-dependent enhancement as a major contributing factor to severe disease (Halstead, 1980; Thein *et al*., 1997). Severe dengue disease, encompasing conditions known as dengue hemorrhagic fever/dengue shock syndrome, involves several well-defined hemostatic abnormalities, including the leakage of

TABLE I
FcRs for Antibody-Enhanced Infection of Dengue Virus

Cell	FcγR[a]	Dengue virus replication		FcγR for ADE
		Ab independent	Ab enhanced	
Monocyte	I,II,III	Yes[b]	Yes[b]	I, II[f]
Dendritic cells	II	Yes[c]	No[c]	None
Mast cell/basophil	I,II	No[d]	Yes[d]	Unknown
Kupffer cell	I,II,III	No[e]	Unknown	Unknown

[a] Compiled from van de Winkel and Anderson (1991), Dijstelbloem *et al*. (2001), Okayama *et al*. (2000), Anselmino *et al*. (1989), and Tuijnman *et al*. (1993).
[b] From Halstead and O'Rourke (1977).
[c] From Wu *et al*. (2000) and Libraty *et al*. (2001).
[d] From King *et al*. (2000).
[e] Abortive infection, but expressing viral antigen (Marianneau *et al*., 1999).
[f] From Littaua *et al*. (1990) and Kontny *et al*. (1988).

plasma into interstitial spaces, as well as thrombocytopenia and bleeding (Halstead, 1990; Kurane *et al*., 1994). The potential to cause severe hemorrhagic disease is a general property of dengue viruses and is not limited to any one viral serotype (Gubler, 1998; Rigau-Perez *et al*., 1998). Although different strains of dengue may influence the severity of hemorrhagic symptoms (Leitmeyer *et al*., 1999; Rico-Hesse *et al*., 1997), it is also generally accepted that pathogenesis depends on immunopathological processes (Rothman and Ennis, 1999). Thus the roles of prior immunity, antibody-enhanced virus infection, and immune-mediated pathologic effects on the vascular system are key points in understanding the pathogenesis of dengue hemorrhagic disease.

While the pathogenesis of severe dengue disease is not completely understood, it is clear from laboratory and epidemiological studies that a considerable risk factor is prior immunity. Severe dengue disease, DHF/DSS, rarely occurs in seronegative individuals suffering their first dengue infection, but instead occurs in individuals who have preexisting dengue viral antibodies, either from a previous infection or from passive antibody transfer, e.g., following maternal transmission of antibodies to the fetus (Kliks *et al*., 1988, 1989). Estimates suggest that 99% of children suffering from DHF/DSS have preexisting immunity from a prior dengue virus infection (Halstead, 1988). Consequently, from this and other studies, it has been calculated that prior exposure to dengue increases the risk for hemorrhagic disease in a

second dengue infection by at least 15-fold (Halstead, 1980; Thein *et al*., 1997). Preexisting serum antibodies can potentiate virus infection by the mechanism of antibody-dependent enhancement, giving rise to amplified virus replication and to an increased potential for the development of hemorrhagic symptoms (Halstead, 1989). Viremic titers are higher in secondary dengue infections in both humans (Gubler *et al*., 1979) and experimental monkeys (Halstead *et al*., 1973). Antibody-enhanced dengue virus infection of human blood monocytes is necessary for the production of endothelial cell activators (Anderson *et al*., 1997), thereby providing a link between antibody-dependent enhancement and alteration of endothelial cell properties, which might contribute to vascular permeability in dengue infection.

For certain other viruses, e.g., influenza (Tamura *et al*., 1993) and HIV (Takeda *et al*., 1990, 1992), distinct "neutralizing" and "antibody-enhancing" epitopes have been identified on the respective viral attachment proteins. Surprisingly, no systematic approach has yet been undertaken to identify regions on the E protein that are essential for ADE, even though this issue was raised as a challenge to research on dengue many years ago (Halstead, 1988).

Human Fcγ receptors are currently categorized into three classes: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). While FcγRI shows high affinity for monomeric IgG, FcγRII and FcγRIII bind monomeric IgG poorly and are more likely involved in binding immune complexes (Dijstelbloem *et al*., 2001). FcγRII is the most widely distributed, being expressed on most circulating leukocytes (van de Winkel and Anderson, 1991). Monocytes express all three FcγRs to varying degrees (van de Winkel and Anderson, 1991), although FcγRI and FcγRII predominate, whereas FcγRIII appears to be limited to a subpopulation ($\sim$10%) of monocytes (Anderson *et al*., 1990; Passlick *et al*., 1989). FcγRIII constitutes the major FcγR on macrophages (Fanger *et al*., 1989), although FcγRI and FcγRII are also present (Tuijnman *et al*., 1993; van de Winkel and Anderson, 1991). It is also important to recognize that FcR expression on cells, including macrophages, can vary depending on the microenvironment (Tomita *et al*., 1994).

Although strong evidence exists for FcγR involvement in ADE of dengue virus, the participating FcγRs *in vivo* have not yet been identified rigorously. In cultured cell lines (monocytic U937 or erythroleukemic K562 cells), FcγRI (Kontny *et al*., 1988) and FcγRII (Littaua *et al*., 1990) have been shown to mediate ADE of dengue virus infection. That FcγRI has the ability to mediate ADE of dengue has been demonstrated using COS cells transfected with FcγRI (Schlesinger and Chapman, 1999).

Dengue and DHF patients show elevated serum levels of interferon (IFN)-γ (Kurane *et al.*, 1991). Because IFN-γ can upregulate both MHC class I and II molecules as well as FcγR (particularly FcγRI) expression in monocytes (Erbe *et al.*, 1990; Perussia *et al.*, 1983), the chances for ADE may be increased, thereby creating a vicious cycle involving positive cytokine feedback and virus amplification (Kurane and Ennis, 1992). IFN-γ has been shown to enhance ADE of dengue virus infection of human monocytic U937 cells (Kontny *et al.*, 1988), although any enhancing effect on dengue infection of peripheral blood monocytes may be negated by the antiviral properties of IFN-γ (Sittisombut *et al.*, 1995).

Mast cells and basophils express mainly FcγRII (Anselmino *et al.*, 1989; Okayama *et al.*, 2001a; Wedi *et al.*, 1996) and some (IFN-γ–inducible) FcγRI (Okayama *et al.*, 2000, 2001b) as well as the high-affinity FcεRI for IgE (Guo *et al.*, 1992; Sperr *et al.*, 1994). As noted previously, the basophil/mast cell KU812 cell line exhibits antibody-enhanced dengue virus infection and produces vasoactive cytokines (King *et al.*, 2000).

Although FcγR-mediated ADE of flaviviruses has been examined extensively as a mechanism for virus amplification, the biological consequences for the participating host cell are not well understood. Because FcγR-mediated cell signaling is complex, the functional effects of virus–antibody interactions with cell surface FcγRs need to be investigated. Monocytes infected with dengue virus in the presence of antibody release cytokines such as TNF-α (Anderson *et al.*, 1997). Induction of TNF-α requires infectious virus (Anderson *et al.*, 1997), suggesting that virus replication (or perhaps expression of one or more crucial viral genes) is responsible for the stimulation of TNF-α release. Therefore, in this case, the FcγR is likely facilitating antibody-enhanced virus replication rather than providing a signal triggered by virus binding to the FcγR. Similarly, antibody-enhanced dengue virus infection of KU812 basophil/mast cells produces IL-1β, IL-6 (King *et al.*, 2000, 2002), and selected chemokines (King *et al.*, 2002). Suppressive effects of antibody-enhanced flavivirus or alphavirus infection on monocyte cytokine secretion have also been reported (Lidbury and Mahalingam, 2000; Yang *et al.*, 2001).

Both activating (FcγRI, FcγRIIa, and FcγRIIIa) and inhibitory (FcγRIIb) forms of FcγRs exist, which mediate signal transduction via a cytoplasmic immunoreceptor tyrosine-based activation motif (ITAM) or inhibitory (ITIM) motif, respectively (Dijstelbloem *et al.*, 2001). The ITAM and associated molecules are necessary for the endocytosis of FcR-bound immune complexes (Amigorena and Bonnerot, 1999) and

therefore play a likely role in the initiating events of antibody-enhanced flavivirus infection. Although not necessary for FcγRII, an accessory subunit (homo- or heterodimeric γ or ζ chains) is required for signaling through FcγRI and FcγRIIIa (Ravetch, 1994). A further FcγR (FcγRIIIb) lacks transmembrane and cytoplasmic domains and is instead anchored to the cell surface membrane via a glycosylphosphatidylinositol (GPI) linkage (Selvaraj *et al*., 1988; Simmons and Seed, 1988). It apparently does not participate in signal transduction and has been speculated to sequester and accumulate immune complexes at specific sites on the cell surface (Huizinga *et al*., 1988; Selvaraj *et al*., 1988).

The roles of activating and inhibitory FcRs in viral ADE have not yet been ascertained. Activating FcRs are expressed on monocytes, macrophages, granulocytes, natural killer (NK) cells, and platelets but not on most lymphocytes (Dijstelbloem *et al*., 2001). Inhibitory FcRs, however, are found on B cells, dendritic cells, and macrophages (Dijstelbloem *et al*., 2001). Interestingly, ADE of dengue virus is best documented for monocytes/macrophages and related cell lines (Halstead, 1989). In contrast, lymphocytic cells (Brandt *et al*., 1979; Kurane *et al*., 1990) and dendritic cells (Wu *et al*., 2000) do not appear to support antibody-enhanced dengue virus infection. Whether this is due to differential expression of activating versus inhibitory FcRs remains to be investigated.

FcRs for IgE (primarily the high-affinity FcεRI) are expressed on cells such as monocytes, macrophages, mast cells, basophils, and dendritic cells and are structurally related to FcγRs (Ravetch, 1994). Their role in binding IgE and/or immune-complexed flaviviruses, such as dengue, remains unexplored. Similarly unexplored is the potential role of the neonatal Fc IgG receptor (FcRn), structurally related to MHC class I and involved in IgG transport across cells (Ghetie and Ward, 2000). In addition to being expressed on certain epithelial and endothelial cells, FcRn is also expressed functionally on monocytes, macrophages, and dendritic cells (Zhu *et al*., 2001).

D. Complement Receptors

In addition to the FcγR, the antibody-complexed flavivirus has been shown to be taken up by a macrophage cell line using the complement receptor-3 (Cardosa *et al*., 1983). In the case studied—West Nile virus infection of mouse P388D1 macrophages—ADE was mediated by the presence of antiviral IgM and was inhibited with a CR3-blocking antibody. This mode of ADE was, however, found to be quantitatively less

productive than the more commonly studied route of ADE, i.e., involving FcγR-mediated uptake route of IgG–virus complexes (Cardosa *et al.*, 1983).

E. Virus Binding Proteins Identified on Cells

The recent demonstration of DC-SIGN as a functional dengue virus receptor on human dendritic cells represents an important advance in the definitive identification of flavivirus receptors (Navarro-Sanchez *et al.*, 2003; Tassaneetrithep *et al.*, 2003). Several studies have identified cell surface proteins that bind flaviviruses, generally assayed by virus overlay blots of SDS–PAGE-resolved cell proteins (Table II). Further work is required to confirm the involvement of these and other proteins as receptors in flavivirus infection.

V. Cell Surface Macromolecules Modulated by Flavivirus Infection

A number of flaviviruses are able to stimulate the expression of cell surface molecules. Notable among these are adhesion molecules and major histocompatibility antigens. Multiple mechanisms appear to be involved, including virus- and cytokine-dependent pathways.

A. MHC Class I

Flavivirus infection of a number of cell types causes an increase in cell surface MHC class I expression (King and Kesson, 1988; King *et al.*, 1989; Libraty *et al.*, 2001; Liu *et al.*, 1989; Lobigs *et al.*, 1996; Shen *et al.*, 1995a, 1997). Evidence for both virus-dependent (Lobigs *et al.*, 1996) and cytokine-dependent (Libraty *et al.*, 2001; Shen *et al.*, 1997) mechanisms has been reported. One process appears to be driven by the amount of flaviviral peptides generated by proteolysis and imported into the transporter associated with antigen processing (TAP), which results in increased cell surface expression of peptide-loaded MHC class I (Momburg *et al.*, 2001). The upregulation of MHC class I molecules by flaviviruses is perhaps reminiscent of that observed in infections by coronaviruses (Suzumura *et al.*, 1986) but stands in contrast to the virus-manipulated downregulation of MHC class I by viruses such as herpesviruses (Jennings *et al.*, 1985; Ploegh, 1998), adenoviruses (Sparer and Gooding, 1998), poxviruses (Boshkov *et al.*, 1992), and HIV (Scheppler *et al.*, 1989). Although enhanced

TABLE II
FLAVIVIRUS BINDING PROTEINS ON CELLS

Cell	Virus	Binding protein(s)	Reference
Human erythroleukemic K562 cells	Dengue-2	100 kDa	Rothwell *et al*. (1996)
Human and mouse neuroblastoma cells	Dengue-2	65 kDa	Ramos-Castaneda *et al*. (1997)
Human monocytic, B and T cell lines	Dengue-2	32, 45, 72 kDa	Bielefeldt-Ohmann (1998); Bieldfeldt-Ohmann *et al*. (2001)
Monkey kidney Vero cells	Dengue-4	44, 74 kDa	Martinez-Barragan and del Angel (2001)
Mosquito C6/36 cells	Dengue-4	40, 45 kDa	Salas-Benito and del Angel (1997)
Mosquito C6/36 cells	Dengue-2	65, 80 kDa	Munoz *et al*. (1998)
Human hepatoma HuH-7 cells	Dengue-1	33- and 37-kDa proteoglycans	Hilgard and Stockert (2000)
Pig kidney PS cells	TBE	35 kDa	Kopecky *et al*. (1999)
Human dendritic cells	Dengue	DC-SIGN	Navarro-Sanchez *et al*. (2003) Tassaneetrithep *et al*. (2003)
Vero cells; mouse neuroblastoma cells	West Nile	105 kDa	Chu and Ng (2003)

MHC class I expression would be expected to lead to greater cytotoxic T (Tc) cell–mediated cytolysis, it would render cells less susceptible to recognition by NK cells. Evidence has been presented that flavivirus-infected cells in fact show reduced susceptibility to NK cells at the cost of enhanced Tc cell–mediated lysis (Lobigs *et al.*, 1996). It has been suggested that such a response may permit flaviviruses to evade an early NK cell response and thereby allow for substantial amplification of virus during the viremic phase of infection (Momburg *et al.*, 2001). Nevertheless, evidence shows that NK cells are activated during dengue infection (Green *et al.*, 1999a), and NK cell–mediated cytotoxicity has been reported to correlate with the severity of disease (Homchampa *et al.*, 1988).

Dendritic cells also undergo upregulation of MHC class I molecules following infection with dengue virus (Libraty *et al.*, 2001). Compared to other antigen-presenting cells, dendritic cells have superior T cell–stimulating activities (McKinney and Streilein, 1989; Timares *et al.*, 1998). Because antigen presentation via dendritic cell MHC class I can provoke exceptionally strong proliferation in CD8-bearing T cells (Bhardwaj *et al.*, 1994; Elbe *et al.*, 1994; McKinney and Streilein, 1989), much of the overall cytotoxic T cell response arising in flavivirus infection may be dictated at the level of the dendritic cell.

B. MHC Class II

West Nile virus infection induces MHC class II expression in mouse macrophages (Shen *et al.*, 1995a), mouse astrocytes (Liu *et al.*, 1989), rat Schwann cells (Argall *et al.*, 1991), and human myoblasts (Bao *et al.*, 1992). Upregulation of dendritic cell MHC class II occurs in response to dengue (Libraty *et al.*, 2001) and West Nile (Johnston *et al.*, 1996) virus infection. Given the potent ability of dendritic cells to activate T cells (Banchereau *et al.*, 2000), the communication between dendritic cell MHC class II–peptide complexes and recognition molecules on CD4-expressing T cells should provide insights into some of the molecular processes underlying T cell activation.

C. Adhesion Molecules

Adhesion molecules are expressed on a variety of cells and mediate a spectrum of processes (Ley, 2001; Roebuck and Finnegan, 1999; Springer, 1995). From the standpoint of flaviviruses, the most significant processes likely concern adhesion molecules on vascular endothelial cells, as these cells regulate permeability as well as

transendothelial migration of leukocytes (Springer, 1995). Of particular importance are intercellular adhesion molecule 1 (ICAM-1; CD54), vascular cell adhesion molecule-1 (VCAM-1; CD106), and E-selectin (CD 62E), which are upregulated on the surface of the endothelium by inflammatory cytokines, cellular stress, and virus infection (Roebuck and Finnegan, 1999).

In the case of dengue, activation of endothelial cells occurs *in vitro* via TNF-α released from antibody-enhanced dengue virus infection of monocytes (Anderson *et al*., 1997). Such activation involves upregulation of adhesion molecules E-selectin, ICAM-1, and VCAM-1. Evidence that similar activation processes occur *in vivo* comes from clinical studies showing elevated serum levels of TNF-α (Green *et al*., 1999b; Hober *et al*., 1993; Vitarana *et al*., 1991; Yadav *et al*., 1991) and soluble VCAM-1 (Murgue *et al*., 2001) in dengue- and DHF/DSS-infected patients. Surprisingly, serum levels of soluble ICAM-1 were actually found to be lower than those of control subjects, although this may reflect plasma protein loss through leakage (Bethell *et al*., 1998). Moreover, the function of soluble forms of ICAM-1 remains unclear, and their expression appeazrs to be regulated differently from that of membrane-bound ICAM-1 (Komatsu *et al*., 1997; van Den Engel *et al*., 2000).

Two phases of ICAM-1 upregulation have been noted in West Nile and Kunjin virus infection of human embryonic fibroblasts, namely an early ($\sim$2 h postinfection) virus-dependent process and a later ($\sim$24 h postinfection) event that is mediated by type 1 interferon (Shen *et al*., 1995b).

For neurotropic flaviviruses, such as West Nile virus in the mouse, the development of encephalitis has been correlated with viremia (Weiner *et al*., 1970), suggesting virus penetration of the blood–brain barrier. The endothelium of the brain microvasculature normally represents a block between circulating virus and the central nervous system. Expression of endothelial cell adhesion molecules, thereby facilitating leukocyte adherence and diapedesis through the endothelium, may be an important mode of dissemination of virus-infected monocytes or other leukocytes into the brain. West Nile virus infection of human endothelial cells causes the upregulation of E-selectin, ICAM-1, and VCAM-1 (Shen *et al*., 1997), which could mediate the transendothelial migration of leukocytes. Upregulation of these adhesion molecules was observed to occur early (2–4 h) in infection and appeared to be triggered by the virus rather than by cytokines (Shen *et al*., 1997).

Further studies are required to clarify the role of endothelial cell adhesion molecule expression in the neuroinvasion of certain flaviviruses. Assuming such a role is confirmed, it will be incumbent to identify the mechanisms by which either free or cell-borne flaviviruses

are stimulated to cross the vascular endothelial layer. For virus-infected leukocytes, such stimulation likely arises, at least in part, from chemokines produced by cells of the central nervous system. Astrocytes infected with JE virus have been reported to release chemokines (RANTES and MCP-1), which may play a role in the transendothelial migration of leukocytes (including those possibly carrying virus) across the blood–brain barrier (Chen *et al*., 2000). Thus, once neural infection is initiated, the process could be amplified by the production of leukocyte-attracting chemokines at the site of infection.

VI. Other Cell Surface Macromolecular Modifications Triggered by Flavivirus Infection

A. Complement Deposition

Complement activation is well documented in dengue disease (Nishioka, 1974; Phanichyakarn *et al*., 1977b; Russell *et al*., 1969), with peak activation and the production of C3a and C5a occurring at the time of vascular leakage and/or shock (Malasit, 1987). Complement activation is likely to be largely mediated by immune complexes consisting of IgG and virus (Bokisch *et al*., 1973a, 1973b; Shaio *et al*., 1992; Sobel *et al*., 1975), although the low levels of circulating immune complexes detected in patients have stimulated thought as to other possible mechanisms (Malasit, 1987). Receptors for C3a and C5a are found on a wide variety of cells, including many human peripheral blood leukocytes (Chenoweth and Hugli, 1978; Fureder *et al*., 1995; Kretzschmar *et al*., 1993; Nilsson *et al*., 1996; van Epps and Chenoweth, 1984). C5a receptors have been reported on endothelial cells, although at lower levels than myeloid cells (Zwirner *et al*., 1999).

Although endothelial cells do not appear to be major targets for dengue virus *in vivo* (Halstead, 1988, 1989; Sahaphong *et al*., 1980), endothelial cells infected with dengue virus *in vitro* can become a substrate for deposition of C3dg and C5b-9, provided the dengue antibody is present (Avirutnan *et al*., 1998). The presence of complement activation products on the endothelial cell surface could be a contributing factor to vascular permeability (Saadi *et al*., 1995). Furthermore, anaphylotoxins and/or deposition of sublytic C5b-9 on the endothelial cell surface has the potential to activate the expression of adhesion molecules (Foreman *et al*., 1994), cytokines (Saadi *et al*., 2000), chemokines (Selvan *et al*., 1998), cyclooxygenase-2

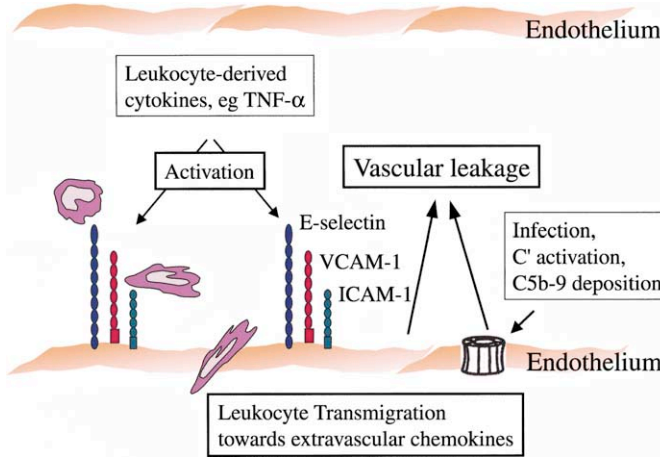

FIG 2. Model depicting possible events in endothelial cell surface perturbation during hemorrhagic flavivirus (dengue) infection. Endothelial cell activation, leading to upregulation of adhesion molecules (E-selectin, VCAM-1, ICAM-1), can be triggered by monocyte-derived cytokines (Anderson *et al.*, 1997) or by deposition of C5b-9 and other products of complement activation (Avirutnan *et al.*, 1998). C5b-9 is represented as a membrane attack complex pore structure, although the deposition of C5b-9 on dengue-infected cells appears associated with sublytic, rather than lytic, responses (Avirutnan *et al.*, 1998). Increased adhesion molecule expression, along with uncharacterized vasoactive factors, can lead to endothelial leakage and can mediate rolling, adhesion, and transendothelial migration of leukocytes into extravascular tissues. Similar processes may also contribute to the invasion of cell-borne neurotropic flaviviruses through the endothelial blood–brain barrier.

(Bustos *et al.*, 1997), tissue factor (Saadi and Platt 1995), heparan sulfate proteoglycan proteinases (Ihrcke and Platt, 1996), and even functional or morphological changes such as permeability loss and gap formation (Saadi *et al.*, 1995).

Thus, in addition to being activated by leukocyte-derived cytokines (Anderson *et al.*, 1997), endothelial cells may also be coaxed toward a more permeability-enhancing state by virus infection and virus-mediated complement deposition. At present, the lack of evidence for *in vivo* infection of endothelial cells by dengue virus would suggest that the cytokine-mediated pathway is dominant. Figure 2 shows a model illustrating the potential role of endothelial cell perturbation by monocyte-derived cytokines and complement activation products in initiating vascular permeability and leukocyte extravasation in severe hemorrhagic flavivirus disease.

VII. Conclusions

Much remains to be learned about the primary receptors for flaviviruses, though much knowledge has been gained about the initial interactions of flaviviruses with cell surface structures. The ability of flaviviruses to affect cell entry through heparan sulfate–type proteoglycans, as well as their dexterity to adjust mutationally to different receptors, depending on host cell type, illustrates the plasticity of the viral E protein to adapt to changing conditions and to ensure successful virus replication. Beyond this, certain flaviviruses, notably dengue virus, are masters at exploiting host antibody and Fc receptor–bearing cells to dramatically amplify viral replication. Flavivirus replication is coupled to altered cellular expression of cytokines, chemokines, and cell surface molecules, which shape the host response and immunopathogenesis associated with flavivirus infections. Ongoing and future characterization of the cell surface structures that mediate these events will be helpful in understanding the mechanisms of flavivirus-induced disease and in developing therapeutic and/or preventive strategies.

Acknowledgments

Research in the author's laboratory was supported by the Canadian Institutes of Health Research and the Natural Sciences and Engineering Research Council of Canada. The author is grateful for the collaboration and advice of many past and present colleagues, including Y. Huang, C. King, A. Issekutz, J. Marshall, C. Osiowy, R. He, S. Wang, B. Innis, A. King, D. Vaughn, K. S. Myint, T. Endy, and M. Mammen.

References

Adelman, B., Sobel, M., Fujimura, Y., Ruggeri, Z. M., and Zimmerman, T. S. (1989). Heparin-associated thrombocytopenia: Observations on the mechanism of platelet aggregation. *J. Lab. Clin. Med.* **113:**204–210.

Ahmad, A., and Menezes, J. (1997). Binding of the Epstein–Barr virus to human platelets causes the release of transforming growth factor-beta. *J. Immunol.* **159:**3984–3988.

Albrecht, P. (1968). Pathogenesis of neurotropic arbovirus infections. *Curr. Top. Microbiol. Immunol.* **43:**44–91.

Allison, S. L., Schalich, J., Stiasny, K., Mandl, C. W., Kunz, C., and Heinz, F. X. (1995). Oligomeric rearrangement of tick-borne encephalitis virus envelope proteins induced by an acidic pH. *J. Virol.* **69:**695–700.

Alving, K. (1991). Airways vasodilation in the immediate allergic reaction: Involvement of inflammatory mediators and sensory nerves. *Acta Physiol. Scand. Suppl.* **597:**1–64.

Amigorena, S., and Bonnerot, C. (1999). Fc receptor signaling and trafficking: A connection for antigen processing. *Immunol. Rev.* **172:**279–284.

Anderson, C. L., Looney, R. J., Culp, D. J., Ryan, D. H., Fleit, H. B., Utell, M. J., Frampton, M. W., Manganiello, P. D., and Guyre, P. M. (1990). Alveolar and peritoneal macrophages bear three distinct classes of Fc receptors for IgG. *J. Immunol.* **145:**196–201.

Anderson, R., King, A. D., and Innis, B. L. (1992). Correlation of E protein binding with cell susceptibility to dengue 4 virus infection. *J. Gen. Virol.* **73:**2155–2159.

Anderson, R., Wang, S., Osiowy, C., and Issekutz, A. C. (1997). Activation of endothelial cells via antibody-enhanced dengue virus infection of peripheral blood monocytes. *J. Virol.* **71:**4226–4232.

Andrews, B. S., Theofilopoulos, A. N., Peters, C. J., Loskutoff, D. J., Brandt, W. E., and Dixon, F. J. (1978). Replication of dengue and Junin viruses in cultured rabbit and human endothelial cells. *Infect. Immun.* **20:**776–781.

Anselmino, L. M., Perussia, B., and Thomas, L. L. (1989). Human basophils selectively express the Fc gamma RII (CDw32) subtype of IgG receptor. *J. Allergy Clin. Immunol.* **84:**907–914.

Anton, F., Morales, C., Aguilar, R., Bellido, C., Aguilar, E., and Gaytan, F. (1998). A comparative study of mast cells and eosinophil leukocytes in the mammalian testis. *Zentralbl. Veterinarmed. A* **45:**209–218.

Argall, K. G., Armati, P. J., King, N. J., and Douglas, M. W. (1991). The effects of West Nile virus on major histocompatibility complex class I and II molecule expression by Lewis rat Schwann cells in vitro. *J. Neuroimmunol.* **35:**273–284.

Aster, R. H. (1989). *In* "Platelet Immunobiology, Molecular and Clinical Aspects" (T. J. Kunicki and G. N. George, eds.), p. 387. Lippincott, Philadelphia.

Avirutnan, P., Malasit, P., Seliger, B., Bhakdi, S., and Husmann, M. (1998). Dengue virus infection of human endothelial cells leads to chemokine production, complement activation, and apoptosis. *J. Immunol.* **161:**6338–6346.

Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y. J., Pulendran, B., and Palucka, K. (2000). Immunobiology of dendritic cells. *Annu. Rev. Immunol.* **18:**767–811.

Bao, S., King, N. J. C., and Dos Remedios, C. G. (1992). Flavivirus induces MHC antigen on human myoblasts: A model of autoimmune myositis. *Muscle Nerve* **15:**1271–1277.

Bayer, W. L., Sherman, F. E., Michaels, R. H., Szeto, I. L. F., and Lewis, J. H. (1965). Purpura in congenital and acquired rubella. *N. Engl. J. Med.* **273:**1362–1366.

Ben-Nathan, D., Kobiler, D., Rzotkiewicz, S., Lustig, S., and Katz, Y. (2000). CNS penetration by noninvasive viruses following inhalational anesthetics. *Ann N. Y. Acad. Sci.* **917:**944–950.

Benyon, R. C., Bissonnette, E. Y., and Befus, A. D. (1991). Tumor necrosis factor-alpha dependent cytotoxicity of human skin mast cells is enhanced by anti-IgE antibodies. *J. Immunol.* **147:**2253–2258.

Bernard, K. A., Klimstra, W. B., and Johnston, R. E. (2000). Mutations in the E2 glycoprotein of Venezuelan equine encephalitis virus confer heparan sulfate interaction, low morbidity, and rapid clearance from blood of mice. *Virology* **276:**93–103.

Berndt, M. C., Chong, B. H., Bull, H. A., Zola, H., and Castaldi, P. A. (1985). Molecular characterization of quinine/quinidine drug-dependent antibody platelet interaction using monoclonal antibodies. *Blood* **66:**1292–1301.

Bethell, D. B., Flobbe, K., Cao, X. T., Day, N. P., Pham, T. P., Buurman, W. A., Cardosa, M. J., White, N. J., and Kwiatkowski, D. (1998). Pathophysiologic and prognostic role of cytokines in dengue hemorrhagic fever. *J. Infect. Dis.* **177:**778–782.

Bevilacqua, M. P. (1993). Endothelial-leukocyte adhesion molecules. *Annu. Rev. Immunol.* **11:**767–804.

Bhamarapravati, N., Tuchinda, P., and Boonyapaknavik, V. (1967). Pathology of Thailand haemorrhagic fever: A study of 100 autopsy cases. *Ann Trop. Med. Parasitol.* **61:**500–510.

Bhardwaj, N., Bender, A., Gonzalez, N., Bui, L. K., Garrett, M. C., and Steinman, R. M. (1994). Influenza virus-infected dendritic cells stimulate strong proliferative and cytolytic responses from human CD8+ T cells. *J. Clin. Invest.* **94:**797–807.

Bielefeldt-Ohmann, H. (1998). Analysis of antibody-independent binding of dengue viruses and dengue virus envelope protein to human myelomonocytic cells and B lymphocytes. *Virus Res.* **57:**63–79.

Bielefeldt-Ohmann, H., Meyer, M., Fitzpatrick, D. R., and Mackenzie, J. S. (2001). Dengue virus binding to human leukocyte cell lines: Receptor usage differs between cell types and virus strains. *Virus Res.* **73:**81–89.

Bik, T., Sarov, I., and Livne, A. (1982). Interaction between vaccinia virus and human blood platelets. *Blood* **59:**482–487.

Birkmann, A., Mahr, K., Ensser, A., Yaguboglu, S., Titgemeyer, F., Fleckenstein, B., and Neipel, F. (2001). Cell surface heparan sulfate is a receptor for human herpesvirus 8 and interacts with envelope glycoprotein K8.1. *J. Virol.* **75:**11583–11593.

Bokisch, V. A., Muller-Eberhard, H. J., and Dixon, F. J. (1973a). The role of complement in hemorrhagic shock syndrome (dengue). *Trans. Assoc. Am. Physicians.* **86:**102–110.

Bokisch, V. A., Top, F. H. J., Russell, P. K., Dixon, F. J., and Muller-Eberhard, H. J. (1973b). The potential pathogenic role of complement in dengue hemorrhagic shock syndrome. *N. Engl. J. Med.* **289:**996–1000.

Boonpucknavig, S., Vuttiviroj, O., Bunnag, C., Bhamarapravati, N., and Nimmanitya, S. (1979). Demonstration of dengue antibody complexes on the surface of platelets from patients with dengue hemorrhagic fever. *Am. J. Trop. Med. Hyg.* **28:**881–884.

Boshkov, L. K., Macen, J. L., and McFadden, G. (1992). Virus-induced loss of class I MHC antigens from the surface of cells infected with myxoma virus and malignant rabbit fibroma virus. *J. Immunol.* **148:**881–887.

Bradding, P., Feather, I. H., Wilson, S., Bardin, P. G., Heusser, C. H., Holgate, S. T., and Howarth, P. H. (1993). Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects: The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J. Immunol.* **151:**3853–3865.

Brandt, W. E., McCown, J. M., Top, F. H., Bancroft, W. H., and Russell, P. K. (1979). Effect of passage history on dengue-2 virus replication in subpopulations of human leukocytes. *Infect. Immun.* **26:**534–541.

Bulger, E. M., and Maier, R. V. (2000). Lipid mediators in the pathophysiology of critical illness. *Crit. Care Med.* **28:**N27–N36.

Bustos, M., Coffman, T. M., Saadi, S., and Platt, J. L. (1997). Modulation of eicosanoid metabolism in endothelial cells in a xenograft model: Role of cyclooxygenase-2. *J. Clin. Invest.* **100:**1150–1158.

Byrnes, A. P., and Griffin, D. E. (2000). Large-plaque mutants of Sindbis virus show reduced binding to heparan sulfate, heightened viremia, and slower clearance from the circulation. *J. Virol.* **74:**644–651.

Cardosa, M. J., Porterfield, J. S., and Gordon, S. (1983). Complement receptor mediates enhanced flavivirus replication in macrophages. *J. Exp. Med.* **158:**258–263.

Cecilia, D., and Ghosh, S. N. (1988). Antibody dependent plaque enhancement by monoclonal antibodies against Japanese encephalitis virus. *Indian J. Med. Res.* **87:**521–525.

Chang, D.-M., and Shaio, M.-F. (1994). Production of interleukin-1 (IL-1) and IL-1 inhibitor by human monocytes exposed to dengue virus. *J. Infect. Dis.* **170:**811–817.

Chaturvedi, U. C., Dhawan, R., Khanna, M., and Mathur, A. (1991). Breakdown of the blood-brain barrier during dengue virus infection of mice. *J. Gen. Virol.* **72:**859–866.

Chen, C. J., Liao, S. L., Kuo, M. D., and Wang, Y. M. (2000). Astrocytic alteration induced by Japanese encephalitis virus infection. *Neuroreport.* **11:**1933–1937.

Chen, Y., Maguire, T., Hileman, R. E., Fromm, J. R., Esko, J. D., Linhardt, R. J., and Marks, R. M. (1997). Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. *Nature Med.* **3:**866–871.

Chen, Y., Maguire, T., and Marks, R. M. (1996). Demonstration of binding of dengue virus envelope protein to target cells. *J. Virol.* **70:**8765–8772.

Chen, Y. C., Wang, S. Y., and King, C. C. (1999). Bacterial lipopolysaccharide inhibits dengue virus infection of primary human monocytes/macrophages by blockade of virus entry via a CD14-dependent mechanism. *J. Virol.* **73:**2650–2657.

Chenoweth, D. E., and Hugli, T. E. (1978). Demonstration of specific C5a receptor on intact human polymorphonuclear leukocytes. *Proc. Natl. Acad. Sci. USA* **75:**3943–3947.

Chong, B. H., Berndt, M. C., Koutts, J., and Castaldi, P. A. (1983). Quinidine-induced thrombocytopenia and leukopenia: Demonstration and characterization of distinct antiplatelet and antileukocyte antibodies. *Blood* **62:**1218–1223.

Chong, B. H., Castaldi, P. A., and Berndt, M. C. (1989a). Heparin-induced thrombocytopenia: Effects of rabbit IgG, and its Fab and FC fragments on antibody-heparin-platelet interaction. *Thromb. Res.* **55:**291–295.

Chong, B. H., Fawaz, I., Chesterman, C. N., and Berndt, M. C. (1989b). Heparin-induced thrombocytopenia: Mechanism of interaction of the heparin-dependent antibody with platelets. *Br. J. Haematol.* **73:**235–240.

Christie, D. J., Mullen, P. C., and Aster, R. H. (1987). Quinine- and quinidine platelet antibodies can react with GPIIb/IIIa. *Br. J. Haematol.* **67:**213–219.

Chu, C. T., Howell, D. N., Morgenlander, J. C., Hulette, C. M., McLendon, R. E., and Miller, S. E. (1999). Electron microscopic diagnosis of human flavivirus encephalitis: Use of confocal microscopy as an aid. *Am. J. Surg. Pathol.* **23:**1217–1226.

Chu, J. J., and Ng, M. L. (2003). Characterization of a 105-kDa plasma membrane associated glycoprotein that is involved in West Nile Virus binding and infection. *Virology* **312:**458–469.

Crill, W. D., and Roehrig, J. T. (2001). Monoclonal antibodies that bind to domain III of dengue virus E glycoprotein are the most efficient blockers of virus adsorption to Vero cells. *J. Virol.* **75:**7769–7773.

Crockett, T. E. (1998). Selectins and mechanisms of signal transduction. *J. Leukocyte Biol.* **63:**1–14.

Danon, D., Jerushalmy, Z., and DeVries, A. (1959). Incorporation of influenza virus in human blood platelets in vitro: Electron microscopical observation. *Virology* **9:**719–722.

Daughaday, C. C., Brandt, W. E., McCown, J. M., and Russell, P. K. (1981). Evidence for two mechanisms of dengue virus infection of adherent human monocytes: Trypsin-sensitive virus receptors and trypsin-resistant immune complex receptors. *Infect. Immun.* **32:**469–473.

Dechecchi, M. C., Melotti, P., Bonizzato, A., Santacatterina, M., Chilosi, M., and Cabrini, G. (2001). Heparan sulfate glycosaminoglycans are receptors sufficient to mediate the initial binding of adenovirus types 2 and 5. *J. Virol.* **75:**8772–8780.

Dechecchi, M. C., Tamanini, A., Bonizzato, A., and Cabrini, G. (2000). Heparan sulfate glycosaminoglycans are involved in adenovirus type 5 and 2-host cell interactions. *Virology* **268:**382–390.

Devine, D. V. (1992). The effects of complement activation on platelets. *Curr. Top. Microbiol. Immunol.* **178:**101–113.

Devine, D. V., and Rosse, W. F. (1995). Identification of platelet proteins that bind alloantibodies and autoantibodies. *Blood* **64:**1240–1245.

Devine, D. V., Siegel, R. S., and Rosse, W. F. (1987). Interactions of the platelets in paroxysmal nocturnal hemoglobinuria with complement: Relationship to defects in the regulation of complement and to platelet survival in vivo. *J. Clin. Invest.* **79:**131–137.

Diamond, G., Legarda, D., and Ryan, L. K. (2000). The innate immune response of the respiratory epithelium. *Immunol. Rev.* **173:**27–38.

Dijstelbloem, H. M., van de Winkel, J. G., and Kallenberg, C. G. (2001). Inflammation in autoimmunity: Receptors for IgG revisited. *Trends. Immunol.* **22:**510–516.

Doerschuk, C. M., Winn, R. K., Coxson, H. O., and Harlan, J. M. (1990). CD18-dependent and -independent mechanisms of neutrophil emigration. *J. Immunol.* **144:**2327–2333.

Doury, J. C., Teyssier, J., Forcain, A., and Doury, F. (1976). Modifications de l'adhesivite plaquettaire au cours de la dengue a form hemorrhagique. *Bull. Soc. Pathol. Exot. Filiales* **69:**493–495.

Dropulic, B., and Masters, C. L. (1990). Entry of neurotropic arboviruses into the central nervous system: An in vitro study using mouse brain endothelium. *J. Infect. Dis.* **161:**685–691.

Duisit, G., Saleun, S., Douthe, S., Barsoum, J., Chadeuf, G., and Moullier, P. (1999). Baculovirus vector requires electrostatic interactions including heparan sulfate for efficient gene transfer in mammalian cells. *J. Gene Med.* **1:**93–102.

Elbe, A., Schleischitz, S., Strunk, D., and Stingl, G. (1994). Fetal skin-derived MHC class I+, MHC class II-dendritic cells stimulate MHC class I-restricted responses of unprimed CD8+ T cells. *J. Immunol.* **153:**2878–2889.

Erbe, D. V., Collins, J. E., Shen, L., Graziano, R. F., and Fanger, M. W. (1990). The effect of cytokines on the expression and function of Fc receptors for IgG on human myeloid cells. *Mol. Immunol.* **27:**57–67.

Fanger, M. W., Shen, L., Graziano, R. F., and Guyre, P. M. (1989). Cytotoxicity mediated by human Fc receptors for IgG. *Immunol. Today.* **10:**92–99.

Feldman, S. A., Audet, S., and Beeler, J. A. (2000). The fusion glycoprotein of human respiratory syncytial virus facilitates virus attachment and infectivity via an interaction with cellular heparan sulfate. *J. Virol.* **74:**6442–6447.

Feldman, S. A., Hendry, R. M., and Beeler, J. A. (1999). Identification of a linear heparin binding domain for human respiratory syncytial virus attachment glycoprotein G. *J. Virol.* **73:**6610–6617.

Ferlenghi, I., Clarke, M., Ruttan, T., Allison, S. L., Schalich, J., Heinz, F. X., Harrison, S. C., Rey, F. A., and Fuller, S. D. (2001). Molecular organization of a recombinant subviral particle from tick-borne encephalitis virus. *Mol. Cell* **7:**593–602.

Fiers, W. (1991). Tumor necrosis factor: Characterization at the molecular, cellular and in vivo level. *FEBS Lett.* **285:**199–212.

Fonseca, B. A. L., Pincus, S., Shope, R. E., Paoletti, E., and Mason, P. W. (1994). Recombinant vaccinia viruses coexpressing dengue-1 glycoproteins prM and E induce neutralizing antibodies in mice. *Vaccine* **12:**279–285.

Foreman, K. E., Vaporciyan, A. A., Bonish, B. K., Jones, M. L., Johnson, K. J., Glovsky, M. M., Eddy, S. M., and Ward, P. A. (1994). C5a-induced expression of P-selectin in endothelial cells. *J. Clin. Invest.* **94:**1147–1155.

Forghani, B., and Schmidt, N. J. (1983). Association of herpes simplex virus with platelets of experimentally infected mice. *Arch. Virol.* **76:**269–274.

Funahara, Y., Ogawa, K., Fujita, N., and Okuno, Y. (1987). Three possible triggers to induce thrombocytopenia in dengue virus infection. *SE. Asian J. Trop. Med. Publ. Hlth* **18:**351–355.

Funk, C. D. (2001). Prostaglandins and leukotrienes: Advances in eicosanoid biology. *Science* **294:**1871–1875.

Fureder, W., Agis, H., Willheim, M., Bankl, H. C., Maier, U., Kishi, K., Muller, M. R., Czerwenka, K., Radaszkiewicz, T., and Butterfield, J. H. (1995). Differential expression of complement receptors on human basophils and mast cells: Evidence for mast cell heterogeneity and CD88/C5aR expression on skin mast cells. *J. Immunol.* **155:**3152–3160.

Galli, S. J., Dvorak, A. M., and Dvorak, H. F. (1984). Basophils and mast cells: Morphologic insights into their biology, secretory patterns, and function. *Prog. Allergy* **34:**1–141.

Gallo, R. L. (2000). Proteoglycans and cutaneous vascular defense and repair. *J. Invest. Dermatol. Symp. Proc.* **5:**55–60.

Gao, J. X., Issekutz, A. C., and Issekutz, T. B. (1994). Neutrophils migrate to delayed-type hypersensitivity reactions in joints but not in skin: Mechanism is LFA-1/MAC-1-independent. *J. Immunol.* **153:**5689–5697.

Gavrilovskaya, I. N., Brown, E. J., Ginsberg, M. H., and Mackow, E. R. (1999). Cellular entry of hantaviruses which cause hemorrhagic fever with renal syndrome is mediated by beta3 integrins. *J. Virol.* **73:**3951–3959.

Ghetie, V., and Ward, E. S. (2000). Multiple roles for the major histocompatibility complex class I-related receptor FcRn. *Annu. Rev. Immunol.* **18:**739–766.

Giroglou, T., Florin, L., Schafer, F., Streeck, R. E., and Sapp, M. (2001). Human papillomavirus infection requires cell surface heparan sulfate. *J. Virol.* **75:**1565–1570.

Goodfellow, I. G., Sioofy, A. B., Powell, R. M., and Evans, D. J. (2001). Echoviruses bind heparan sulfate at the cell surface. *J. Virol.* **75:**4918–4921.

Grabbe, J., Welker, P., Moller, A., Dippel, E., Ashman, L. K., and Czarnetzki, B. M. (1994). Comparative cytokine release from human monocytes, monocyte-derived immature mast cells, and a human mast cell line (HMC-1). *J. Invest. Dermatol.* **103:**504–508.

Green, S., Pichyangkul, S., Vaughan, D. W., Kalayanarooj, S., Nimmannitya, S., Nisalak, A., Kurane, I., Rothman, A. L., and Ennis, F. A. (1999a). Early CD69 expression on peripheral blood lymphocytes from children with dengue hemorrhagic fever. *J. Infect Dis.* **180:**1429–1435.

Green, S., Vaughn, D. W., Kalayanarooj, S., Nimmannitya, S., Suntayakorn, S., Nisalak, A., Lew, R., Innis, B. L., Kurane, I., Rothman, A. L., and Ennis, F. A. (1999b). Early immune activation in acute dengue illness is related to development of plasma leakage and disease severity. *J. Infect. Dis.* **179:**755–762.

Gubler, D. J. (1988). *In* "The Arboviruses: Epidemiology and Ecology" (T. P. Monath, ed.), Vol. II, pp. 223–260. CRC, Boca Raton, FL.

Gubler, D. J. (1998). Dengue and dengue hemorrhagic fever. *Clin. Microbiol. Rev.* **11:**480–496.

Gubler, D. J., Suharyono, W., Lubis, I., Eram, S., and Sulianti, S. J. (1979). Epidemic dengue hemorrhagic fever in rural Indonesia: I. Virological and epidemiological studies. *Am. J. Trop. Med. Hyg.* **28:**701–710.

Guirakhoo, F., Bolin, R. A., and Roehrig, J. T. (1992). The Murray Valley encephalitis virus prM protein confers acid resistance to virus particles and alters the expression of epitopes within the R2 domain of E glycoprotein. *Virology* **191:**921–931.

Guirakhoo, F., Heinz, F. X., Mandl, C. W., Holzmann, H., and Kunz, C. (1991). Fusion activity of flaviviruses: Comparison of mature and immature (prM-containing) tick-borne encephalitis virions. *J. Gen. Virol.* **72:**1323–1329.

Guo, C. B., Kagey-Sobotka, A., Lichtenstein, L. M., and Bochner, B. S. (1992). Immunophenotyping and functional analysis of purified human uterine mast cells. *Blood* **79:**708–712.

Hackett, T., Kelton, J. G., and Powers, P. (1982). Drug-induced platelet destruction. *Semin. Thromb. Hemost.* **8:**116–137.

Hall, W. C., Crowell, T. P., Watts, D. M., Barros, V. L., Kruger, H., Pinheiro, F., and Peters, C. J. (1991). Demonstration of yellow fever and dengue antigens in formalin-fixed paraffin-embedded human liver by immunohistochemical analysis. *Am. J. Trop. Med. Hyg.* **45:**408–417.

Halstead, S. B. (1980). *In* "The togaviruses: Biology, Structure, Replication" (R. W. Schlesinger, ed.), pp. 107–173. Academic Press, New York.

Halstead, S. B. (1982). Immune enhancement of viral infection. *Prog. Allergy* **31:**301–364.

Halstead, S. B. (1988). Pathogenesis of dengue: Challenges to molecular biology. *Science* **239:**476–481.

Halstead, S. B. (1989). Antibody, macrophages, dengue virus infection, shock, and hemorrhage: A pathogenetic cascade. *Rev. Infect. Dis.* **11**(Suppl. 4):S830–S839.

Halstead, S. B. (1990). *In* "Tropical and Geographical Medicine" (K. S. Warren and A. A. F. Mahmoud, eds.), 2nd Ed., pp. 675–685. McGraw-Hill, New York.

Halstead, S. B., Diwan, A. R., Marchette, N. J., Palumbo, N. E., and Srisukonth, L. (1984a). Selection of attenuated dengue 4 viruses by serial passage in primary kidney cells: I. Attributes of uncloned virus at different passage levels. *Am. J. Trop. Med. Hyg.* **33:**654–665.

Halstead, S. B., Marchette, N. J., Diwan, A. R., Palumbo, N. E., and Putvatana, R. (1984b). Selection of attenuated dengue 4 viruses by serial passage in primary kidney cells: II. Attributes of virus cloned at different dog kidney passage levels. *Am. J. Trop. Med. Hyg.* **33:**666–671.

Halstead, S. B., Marchette, N. J., Diwan, A. R., Palumbo, N. E., Putvatana, R., and Larsen, L. K. (1984c). Selection of attenuated dengue 4 viruses by serial passage in primary kidney cells: III. Reversion to virulence by passage of cloned virus in fetal rhesus lung cells. *Am. J. Trop. Med. Hyg.* **33:**672–678.

Halstead, S. B., and O'Rourke, E. J. (1977). Dengue viruses and mononuclear phagocytes: I. Infection enhancement by non-neutralizing antibody. *J. Exp. Med.* **146:**201–217.

Halstead, S. B., O'Rourke, E. J., and Allison, A. C. (1977). Dengue viruses and mononuclear phagocytes: II. Identity of blood and tissue leukocytes supporting in vitro infection. *J. Exp. Med.* **146:**218–229.

Halstead, S. B., Porterfield, J. S., and O'Rourke, E. J. (1980). Enhancement of dengue virus infection in monocytes by flavivirus antisera. *Am. J. Trop. Med. Hyg.* **29:**638–642.

Halstead, S. B., Shotwell, H., and Casals, J. (1973). Studies on the pathogenesis of dengue infection in monkeys: II. Clinical laboratory responses to heterologous infection. *J. Infect. Dis.* **128:**15–22.

He, R. T., Innis, B. L., Nisalak, A., Usawattanakul, W., Wang, S., Kalayanarooj, S., and Anderson, R. (1995). Antibodies that block virus attachment to Vero cells are a major component of the human neutralizing antibody response against dengue virus type 2. *J. Med. Virol.* **45:**451–461.

Heil, M. L., Albee, A., Strauss, J. H., and Kuhn, R. J. (2001). An amino acid substitution in the coding region of the E2 glycoprotein adapts Ross River virus to utilize heparan sulfate as an attachment moiety. *J. Virol.* **75:**6303–6309.

Heinz, F. X., and Allison, S. L. (2001). The machinery for flavivirus fusion with host cell membranes. *Curr. Opin. Microbiol.* **4:**450–455.

Heinz, F. X., Allison, S. L., Stiasny, K., Schalich, J., Holzmann, H., Mandl, C. W., and Kunz, C. (1995). Recombinant and virion-derived soluble and particulate immunogens for vaccination against tick-borne encephalitis. *Vaccine* **13:**1636–1642.

Heinz, F. X., Stiasny, K., Puschner-Auer, G., Holzmann, H., Allison, S. L., Mandl, C. W., and Kunz, C. (1994). Structural changes and functional control of the tick-borne encephalitis virus glycoprotein E by the heterodimeric association with protein prM. *Virology* **198:**109–117.

Hilgard, P., and Stockert, R. (2000). Heparan sulfate proteoglycans initiate dengue virus infection of hepatocytes. *Hepatology* **32:**1069–1077.

Hiramatsu, K., Tadano, M., Men, R., and Lai, C.-J. (1996). Mutational analysis of a neutralization epitope on the dengue type 2 virus (DEN2) envelope protein: Monoclonal antibody resistant DEN2/DEN4 chimeras exhibit reduced mouse neurovirulence. *Virology* **224:**437–445.

Hober, D., Poli, L., Roblin, B., Gestas, P., Chungue, E., Granic, G., Imbert, P., Pecarere, J. L., Vergez-Pascal, R., Wattre, P., and Maniez-Montreuil, M. (1993). Serum levels of tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6) and interleukin-1 beta (IL-1 beta) in dengue-infected patients. *Am. J. Trop. Med. Hyg.* **48:**324–331.

Holbrook, M. R., Ni, H., Shope, R. E., and Barrett, A. D. (2001). Amino acid substitution(s) in the stem-anchor region of langat virus envelope protein attenuates mouse neurovirulence. *Virology* **286:**54–61.

Homchampa, P., Sarasombath, S., Suvatte, V., and Vongskul, M. (1988). Natural killer cells in dengue hemorrhagic fever/dengue shock syndrome. *Asian Pac. J. Allergy Immunol.* **6:**95–102.

Hsiao, J. C., Chung, C. S., and Chang, W. (1999). Vaccinia virus envelope D8L protein binds to cell surface chondroitin sulfate and mediates the adsorption of intracellular mature virions to cells. *J. Virol.* **73:**8750–8761.

Huerre, M. R., Lan, N. T., Marianneau, P., Hue, N. B., Khun, H., Hung, N. T., Khen, N. T., Drouet, M. T., Huong, V. T., Ha, D. Q., Buisson, Y., and Deubel, V. (2001). Liver histopathology and biological correlates in five cases of fatal dengue fever in Vietnamese children. *Virch. Arch.* **438:**107–115.

Huizinga, T. W. J., van der Schoot, C. E., Jost, C., Klaassen, R., Kleijer, M., Von dem Borne, A. E. G. K., Roos, D., and Tetteroo, P. A. T. (1988). The PI-linked receptor FcRIII is released on stimulation of neutrophils. *Nature* **333:**667–669.

Hulst, M. M., van Gennip, H. G., and Moormann, R. J. (2000). Passage of classical swine fever virus in cultured swine kidney cells selects virus variants that bind to heparan sulfate due to a single amino acid change in envelope protein E(rns). *J. Virol.* **74:**9553–9561.

Hulst, M. M., van Gennip, H. G., Vlot, A. C., Schooten, E., de Smit, A. J., and Moormann, R. J. (2001). Interaction of classical swine fever virus with membrane-associated heparan sulfate: Role for virus replication in vivo and virulence. *J. Virol.* **75:**9585–9595.

Hurrelbrink, R. J., and McMinn, P. C. (2001). Attenuation of Murray Valley encephalitis virus by site-directed mutagenesis of the hinge and putative receptor-binding regions of the envelope protein. *J. Virol.* **75:**7692–7702.

Ihrcke, N. S., and Platt, J. L. (1996). Shedding of heparan sulfate proteoglycan by stimulated endothelial cells: Evidence for proteolysis of cell-surface molecules. *J. Cell Physiol.* **168:**625–637.

Imbach, P. A. (1994). Harmful and beneficial antibodies in immune thrombocytopenic purpura. *Clin. Exp. Immunol.* **97**(Suppl 1):25–30.

Imler, J. L., and Hoffmann, J. A. (2000). Toll and Toll-like proteins: An ancient family of receptors signaling infection. *Rev. Immunogenet.* **2:**294–304.

Issekutz, A. C., Chuluyan, H. E., and Lopes, N. (1995). CD11/CD18-independent transendothelial migration of human polymorphonuclear leokocytes and monocytes: Involvement of distinct and unique mechanisms. *J. Leukocyte. Biol.* **57:**553–561.

Issekutz, A. C., and Issekutz, T. B. (1993). A major portion of polymorphonuclear leukocyte and T lymphocyte migration to arthritis in the rat is via LFA-1/MAC-1 independent mechanisms. *Clin. Immunol. Immunopathol.* **67:**257–263.

Jennings, S. R., Rice, P. L., Kloszewski, E. D., Anderson, R. W., Thompson, D. L., and Tevethia, S. S. (1985). Effect of herpes simplex virus types 1 and 2 on surface expression of class I major histocompatibility complex antigens on infected cells. *J. Virol.* **56:**757–766.

Johnston, L. J., Halliday, G. M., and King, N. J. (1996). Phenotypic changes in Langerhans' cells after infection with arboviruses: A role in the immune response to epidermally acquired viral infection? *J. Virol.* **70:**4761–4766.

Johnston, L. J., Halliday, G. M., and King, N. J. (2000). Langerhans cells migrate to local lymph nodes following cutaneous infection with an arbovirus. *J. Invest. Dermatol.* **114:**560–568.

Kelton, J. G., Sheridan, D., Santos, A., Smith, J., Steeves, K., Smith, C., Brown, C., and Murphy, W. G. (1988). Heparin-induced thrombocytopenia: Laboratory studies. *Blood* **72:**925–930.

Killen, H., and O'Sullivan, M. A. (1993). Detection of dengue virus by in situ hybridization. *J. Virol. Methods* **41:**135–146.

King, A. D., Nisalak, A., Kalayanrooj, S., Myint, K. A., Pattanapanyasat, K., Nimmannitya, S., and Innis, B. L. (1999). B cells are the principal circulating mononuclear cells infected by dengue virus. *SE. Asian J. Trop. Med. Public Health* **30:**718–728.

King, C., Anderson, R., and Marshall, J. S. (2002). Dengue virus selectively induces human mast cell chemokine production. *J. Virol.* **76:**8408–8419.

King, C. A., Marshall, J. S., Alshurafa, H., and Anderson, R. (2000). Release of vasoactive cytokines by antibody-enhanced dengue virus infection of a human mast cell/basophil line. *J. Virol.* **74:**7146–7150.

King, N. J., and Kesson, A. M. (1988). Interferon-independent increases in class I major histocompatibility complex antigen expression follow flavivirus infection. *J. Gen. Virol.* **69:**2535–2543.

King, N. J., Maxwell, L. E., and Kesson, A. M. (1989). Induction of class I major histocompatibility complex antigen expression by West Nile virus on gamma interferon-refractory early murine trophoblast cells. *Proc. Natl. Acad. Sci. USA* **86:**911–915.

Kliks, S. C., Nimmanitya, S., Nisalak, A., and Burke, D. S. (1988). Evidence that maternal dengue antibodies are important in the development of dengue hemorrhagic fever in infants. *Am. J. Trop. Med. Hyg.* **38:**411–419.

Kliks, S. C., Nisalak, A., Brandt, W. E., Wahl, L., and Burke, D. S. (1989). Antibody-dependent enhancement of dengue virus growth in human monocytes as a risk factor for dengue hemorrhagic fever. *Am. J. Trop. Med. Hyg.* **40:**444–451.

Klimstra, W. B., Ryman, K. D., Bernard, K. A., Nguyen, K. B., Biron, C. A., and Johnston, R. E. (1999). Infection of neonatal mice with sindbis virus results in a systemic inflammatory response syndrome. *J. Virol.* **73:**10387–10398.

Klimstra, W. B., Ryman, K. D., and Johnston, R. E. (1998). Adaptation of Sindbis virus to BHK cells selects for use of heparan sulfate as an attachment receptor. *J. Virol.* **72:**7357–7366.

Komatsu, S., Flores, S., Gerritsen, M. E., Anderson, D. C., and Granger, D. N. (1997). Differential up-regulation of circulating soluble and endothelial cell intercellular adhesion molecule-1 in mice. *Am. J. Pathol.* **151:**205–214.

Kontny, U., Kurane, I., and Ennis, F. A. (1988). Gamma interferon augments Fcγ receptor-mediated dengue virus infection of human monocytic cells. *J. Virol.* **62:**3928–3933.

Kopecky, J., Grubhoffer, L., Kovar, V., Jindrak, L., and Vokurkova, D. (1999). A putative host cell receptor for tick-borne encephalitis virus identified by anti-idiotypic antibodies and virus affinoblotting. *Intervirology* **42:**9–16.

Kownatzki, E. (1982). Triggering of mast cells. *Mol. Immunol.* **19:**1297–1300.

Kretzschmar, T., Jeromin, A., Gietz, C., Bautsch, W., Klos, A., Kohl, J., Rechkemmer, G., and Bitter-Suermann, D. (1993). Chronic myelogenous leukemia-derived basophilic granulocytes express a functional active receptor for the anaphylatoxin C3a. *Eur. J. Immunol.* **23:**558–561.

Krishnamurti, C., Kalayanarooj, S., Cutting, M. A., Peat, R. A., Rothwell, S. W., Reid, T. J., Green, S., Nisalak, A., Endy, T. P., Vaughn, D. W., Nimmannitya, S., and Innis, B. L. (2001). Mechanisms of hemorrhage in dengue without circulatory collapse. *Am. J. Trop. Med. Hyg.* **65:**840–847.

Kurane, I., and Ennis, F. E. (1992). Immunity and immunopathology in dengue virus infections. *Semin. Immuno.* **4:**121–127.

Kurane, I., Innis, B. L., Nimmannitya, S., Nisalak, A., Meager, A., Janus, J., and Ennis, F. A. (1991). Activation of T lymphocytes in dengue virus infections: High levels of soluble interleukin 2 receptor, soluble CD4, soluble CD8, interleukin 2, and interferon-gamma in sera of children with dengue. *J. Clin. Invest.* **88:**1473–1480.

Kurane, I., Kontny, U., Janus, J., and Ennis, F. A. (1990). Dengue-2 virus infection of human mononuclear cell lines and establishment of persistent infections. *Arch. Virol.* **110:**91–101.

Kurane, I., Rothman, A. L., Livingston, P. G., Green, S., Gagnon, S. J., Janus, J., Innis, B. L., Nimmannitya, S., Nisalak, A., and Ennis, F. A. (1994). Immunopathologic mechanisms of dengue hemorrhagic fever and dengue shock syndrome. *Arch. Virol.* **136**(Suppl. 9):59–64.

Kurt-Jones, E. A., Popova, L., Kwinn, L., Haynes, L. M., Jones, L. P., Tripp, R. A., Walsh, E. E., Freeman, M. W., Golenbock, D. T., Anderson, L. J., and Finberg, R. W. (2000). Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nature Immunol.* **1:**398–401.

Laberge, S., Rabb, H., Issekutz, T., and Martin, J. G. (1995). Role of VLA-4 and LFA-1 in allergen-induced airway inflammation in the rat. *Am. J. Resp. Crit. Care Med.* **151:**822–829.

Larke, R. P. B., and Wheelock, F. (1970). Stabilization of chikungunya virus infectivity by human blood platelets. *J. Infect. Dis.* **122:**523–531.

Lee, E., and Lobigs, M. (2000). Substitutions at the putative receptor-binding site of an encephalitic flavivirus alter virulence and host cell tropism and reveal a role for glycosaminoglycans in entry. *J. Virol.* **74:**8867–8875.

Lee, E., Weir, R. C., and Dalgarno, L. (1997). Changes in the dengue virus major envelope protein on passaging and their localization on the three-dimensional structure of the protein. *Virology* **232:**281–290.

Lee, T.-H., Stromberg, R. R., Henrard, D., and Busch, M. P. (1993). Effect of platelet-associated virus on assays of HIV-1 in plasma. *Science* **262:**1585–1585.

Lefer, A. M. (1989). Trends in shock research: Significance of lipid mediators in shock states. *Circ. Shock* **27:**3–12.

Leitmeyer, K. C., Vaughn, D. W., Watts, D. M., Salas, R., Villalobos, I., de, C., Ramos, C., and Rico-Hesse, R. (1999). Dengue virus structural differences that correlate with pathogenesis. *J. Virol.* **73:**4738–4747.

Lescar, J., Roussel, A., Wien, M. W., Navaza, J., Fuller, S. D., Wengler, G., and Rey, F. A. (2001). The fusion glycoprotein shell of Semliki Forest virus: An icosahedral assembly primed for fusogenic activation at endosomal pH. *Cell* **105:**137–148.

Ley, K. (2001). Pathways and bottlenecks in the web of inflammatory adhesion molecules and chemoattractants. *Immunol. Res.* **24:**87–95.

Libraty, D. H., Pichyangkul, S., Ajariyakhajorn, C., Endy, T. P., and Ennis, F. A. (2001). Human dendritic cells are activated by dengue virus infection: Enhancement by gamma interferon and implications for disease pathogenesis. *J. Virol.* **75:**3501–3508.

Lidbury, B. A., and Mahalingam, S. (2000). Specific ablation of antiviral gene expression in macrophages by antibody-dependent enhancement of Ross River virus infection. *J. Virol.* **74:**8376–8381.

Lin, C. F., Lei, H. Y., Liu, C. C., Liu, H. S., Yeh, T. M., Wang, S. T., Yang, T. I., Sheu, F. C., Kuo, C. F., and Lin, Y. S. (2001). Generation of IgM anti-platelet autoantibody in dengue patients. *J. Med. Virol.* **63:**143–149.

Lin, C. L., Chung, C. S., Heine, H. G., and Chang, W. (2000). Vaccinia virus envelope H3L protein binds to cell surface heparan sulfate and is important for intracellular mature virion morphogenesis and virus infection in vitro and in vivo. *J. Virol.* **74:**3353–3365.

Liou, M. L., and Hsu, C. Y. (1998). Japanese encephalitis virus is transported across the cerebral blood vessels by endocytosis in mouse brain. *Cell Tissue Res.* **293:**389–394.

Littaua, R., Kurane, I., and Ennis, F. A. (1990). Human IgG Fc receptor II mediates antibody-dependent enhancement of dengue virus infection. *J. Immunol.* **144:**3183–3186.

Liu, J., and Thorp, S. C. (2002). Cell surface heparan sulfate and its roles in assisting viral infections. *Med. Res. Rev.* **22:**1–25.

Liu, Y., King, N., Kesson, A., Blanden, R. V., and Mullbacher, A. (1989). Flavivirus infection up-regulates the expression of class I and class II major histocompatibility antigens on and enhances T cell recognition of astrocytes in vitro. *J. Neuroimmunol.* **21:**157–168.

Lobigs, M., Blanden, R. V., and Mullbacher, A. (1996). Flavivirus-induced up-regulation of MHC class I antigens: Implications for the induction of CD8+ T-cell-mediated autoimmunity. *Immunol. Rev.* **152:**5–19.

Lobigs, M., Usha, R., Nestorowicz, A., Marshall, I. D., Weir, R. C., and Dalgarno, L. (1990). Host cell selection of Murray Valley encephalitis virus variants altered at an RGD sequence in the envelope protein and in mouse virulence. *Virology* **176:**587–595.

Lowell, C. A., and Berton, G. (1999). Integrin signal transduction in myeloid leukocytes. *J. Leukocyk Biol.* **65:**313–320.

Luscinskas, F. W., Cybulsky, M. I., Kiely, J. M., Peckins, C. S., Davis, V. M., and Gimbrone-MA, J. (1991). Cytokine-activated human endothelial monolayers support enhanced neutrophil transmigration via a mechanism involving both

endothelial-leukocyte adhesion molecule-1 and intercellular adhesion molecule-1. *J. Immunol.* **146:**1617–1625.

Lustig, S., Danenberg, H. D., Kafri, Y., Kobiler, D., and Ben-Nathan, D. (1992). Viral neuroinvasion and encephalitis induced by lipopolysaccharide and its mediators. *J. Exp. Med.* **176:**707–712.

Malasit, P. (1987). Complement and dengue hemorrhagic fever/shock syndrome. *SE. Asian J. Trop. Med. Publ. Hlth.* **18:**316–320.

Mandl, C. W., Allison, S. L., Holzmann, H., Meixner, T., and Heinz, F. X. (2000). Attenuation of tick-borne encephalitis virus by structure-based site-specific mutagenesis of a putative flavivirus receptor binding site. *J. Virol.* **74:**9601–9609.

Mandl, C. W., Kroschewski, H., Allison, S. L., Kofler, R., Holzmann, H., Meixner, T., and Heinz, F. X. (2001). Adaptation of tick-borne encephalitis virus to BHK-21 cells results in the formation of multiple heparan sulfate binding sites in the envelope protein and attenuation in vivo. *J. Virol.* **75:**5627–5637.

Marchette, N. J., and Halstead, S. B. (1978). Phytohemagglutinin enhancement of dengue-2 virus replication in nonimmune rhesus monkey peripheral blood leukocytes. *Infect. Immun.* **19:**40–45.

Marianneau, P., Steffan, A. M., Royer, C., Drouet, M. T., Jaeck, D., Kirn, A., and Deubel, V. (1999). Infection of primary cultures of human Kupffer cells by Dengue virus: No viral progeny synthesis, but cytokine production is evident. *J. Virol.* **73:**5201–5206.

Marks, R. M., Lu, H., Sundaresan, R., Toida, T., Suzuki, A., Imanari, T., Hernaiz, M. J., and Linhardt, R. J. (2001). Probing the interaction of dengue virus envelope protein with heparin: Assessment of glycosaminoglycan-derived inhibitors. *J. Med. Chem.* **44:**2178–2187.

Marshall, J. S., and Bienenstock, J. (1994). The role of mast cells in inflammatory reactions of the airways, skin and intestine. *Curr. Opin. Immunol.* **6:**853–859.

Marshall, J. S., Ford, G. P., and Bell, E. B. (1987). Formalin sensitivity and differential staining of mast cells in human dermis. *Br. J. Dermatol.* **117:**29–36.

Martinez-Barragan, J. J., and del Angel, R. M. (2001). Identification of a putative coreceptor on vero cells that participates in dengue 4 virus infection. *J. Virol.* **75:**7818–7827.

Maruo, N., Morita, I., Shirao, M., and Murota, S. I. (1992). IL-6 increases endothelial permeability in vitro. *Endocrinology* **131:**710.

Mason, P. W., Pincus, S., Fournier, M. J., Mason, T. L., Shope, R. E., and Paoletti, E. (1991). Japanese encephalitis virus-vaccinia recombinants produce particulate forms of the structural membrane proteins and induce high levels of protection against lethal JEV infection. *Virology* **180:**294–305.

Matthews, V., Robertson, T., Kendrick, T., Abdo, M., Papadimitriou, J., and McMinn, P. (2000). Morphological features of Murray Valley encephalitis virus infection in the central nervous system of Swiss mice. *Int. J. Exp. Pathol.* **81:**31–40.

McKinney, E. C., and Streilein, J. W. (1989). On the extraordinary capacity of allogeneic epidermal Langerhans cells to prime cytotoxic T cells in vivo. *J. Immunol.* **143:**1560–1564.

McMinn, P. C., Dalgarno, L., and Weir, R. C. (1996). A comparison of the spread of Murray Valley encephalitis viruses of high or low neuroinvasiveness in the tissues of Swiss mice after peripheral inoculation. *Virology* **220:**414–423.

Megret, F., Hugnot, J. P., Falconar, A., Gentry, M. K., Morens, D. M., Murray, J. M., Schlesinger, J. J., Wright, P. J., Young, P., Van Regenmortel, M. H. V., and Deubel, V. (1992). Use of recombinant fusion proteins and monoclonal antibodies to define linear

and discontinuous antigenic sites on the dengue virus envelope glycoprotein. *Virology* **187:**480–491.

Mentor, N. A., and Kurane, I. (1997). Dengue virus infection of human T lymphocytes. *Acta Virol.* **41:**175–176.

Michel, C. C. (1988). Capillary permeability and how it may change. *J. Physiol.* **404:**1–29.

Mitrakul, C., Poshyachinda, M., Futrakul, P., Sangkawibha, N., and Ahandrik, S. (1977). Hemostatic and platelet kinetic studies in dengue hemorrhagic fever. *Am. J. Trop. Med. Hyg.* **26:**975–984.

Moller, A., Grabbe, J., and Czarnetzki, B. M. (1991). Mast cells and their mediators in immediate and delayed immune reactions. *Skin Pharmacol.* **4**(Suppl 1):56–63.

Moller, A., Henz, B. M., Grutzkau, A., Lippert, U., Aragane, Y., Schwarz, T., and Kruger, K. S. (1998). Comparative cytokine gene expression: Regulation and release by human mast cells. *Immunology* **93:**289–295.

Moller, A., Lippert, U., Lessmann, D., Kolde, G., Hamann, K., Welker, P., Schadendorf, D., Rosenbach, T., Luger, T., and Czarnetzki, B. M. (1993). Human mast cells produce IL-8. *J. Immunol.* **151:**3261–3266.

Momburg, F., Mullbacher, A., and Lobigs, M. (2001). Modulation of transporter associated with antigen processing (TAP)-mediated peptide import into the endoplasmic reticulum by flavivirus infection. *J. Virol.* **75:**5663–5671.

Monath, T. P. (1994). Dengue: The risk to developed and developing countries. *Proc. Natl. Acad. Sci. USA* **91:**2395–2400.

Monath, T. P., Arroyo, J., Levenbook, I., Zhang, Z. X., Catalan, J., Draper, K., and Guirakhoo, F. (2002). Single mutation in the flavivirus envelope protein hinge region increases neurovirulence for mice and monkeys but decreases viscerotropism for monkeys: Relevance to development and safety testing of live, attenuated vaccines. *J. Virol.* **76:**1932–1943.

Monath, T. P., Ballinger, M. E., Miller, B. R., and Salaun, J. J. (1989). Detection of yellow fever viral RNA by nucleic acid hybridization and viral antigen by immunocytochemistry in fixed human liver. *Am. J. Trop. Med. Hyg.* **40:**663–668.

Monath, T. P., Cropp, C. B., and Harrison, A. K. (1983). Mode of entry of a neurotropic arbovirus into the central nervous system: Reinvestigation of an old controversy. *Lab. Invest.* **48:**399–410.

Montrucchio, G., Alloatti, G., and Camussi, G. (2000). Role of platelet-activating factor in cardiovascular pathophysiology. *Physiol. Rev.* **80:**1669–1699.

Morzycki, W., Sadowska, J., and Issekutz, A. C. (1990). Interleukin-1 and tumour necrosis factor alpha induced polymorphonuclear leukocyte-endothelial cell adhesion and transendothelial migration in vitro: The effect of apical versus basal monolayer stimulation. *Immunol. Lett.* **25:**331–340.

Moser, R., Schleiffenbaum, B., Groscurth, P., and Fehr, J. (1989). Interleukin 1 and tumor necrosis factor stimulate human vascular endothelial cells to promote transendothelial neutrophil passage. *J. Clin. Invest.* **83:**444–455.

Munoz, M. L., Cisneros, A., Cruz, J., Das, P., Tovar, R., and Ortega, A. (1998). Putative dengue virus receptors from mosquito cells. *FEMS Microbiol. Lett.* **168:**251–258.

Murgue, B., Cassar, O., and Deparis, X. (2001). Plasma concentrations of sVCAM-1 and severity of dengue infections. *J. Med. Virol.* **65:**97–104.

Navarro-Sanchez, E., Altmeyer, R., Amara, A., Schwartz, O., Fieschi, F., Virelizier, J. L., Arenzana-Seisdedos, F., and Desprès, P. (2003). Dendritic-cell-specific ICAM3-grabbing non-integrin is essential for the productive infecton of human dendritic cells by mosquito-cell-derived dengue viruses. *EMBO Rep.* **4**(7 Suppl):1–6.

Neff, S., Sa-Carvalho, D., Rieder, E., Mason, P. W., Blystone, S. D., Brown, E. J., and Baxt, B. (1998). Foot-and-mouth disease virus virulent for cattle utilizes the integrin alpha(v)beta3 as its receptor. *J. Virol.* **72:**3587–3594.

Ni, H., and Barrett, A. D. (1998). Attenuation of Japanese encephalitis virus by selection of its mouse brain membrane receptor preparation escape variants. *Virology* **241:**30–36.

Ni, H., Ryman, K. D., Wang, H., Saeed, M. F., Hull, R., Wood, D., Minor, P. D., Watowich, S. J., and Barrett, A. D. (2000). Interaction of yellow fever virus French neurotropic vaccine strain with monkey brain: Characterization of monkey brain membrane receptor escape variants. *J. Virol.* **74:**2903–2906.

Nilsson, G., Johnell, M., Hammer, C. H., Tiffany, H. L., Nilsson, K., Metcalfe, D. D., Siegbahn, A., and Murphy, P. M. (1996). C3a and C5a are chemotaxins for human mast cells and act through distinct receptors via a pertussis toxin-sensitive signal transduction pathway. *J. Immunol.* **157:**1693–1698.

Nilsson, G., Svensson, V., and Nilsson, K. (1995). Constitutive and inducible cytokine mRNA expression in the human mast cell line HMC-1. *Scand. J. Immunol.* **42:**76–81.

Nishioka, K. (1974). Serum complement level in dengue hemorrhagic fever. *Allerg. Immunol. (Leipz.)* **20–21:**385–392.

Okayama, Y., Hagaman, D. D., and Metcalfe, D. D. (2001a). A comparison of mediators released or generated by IFN-gamma–treated human mast cells following aggregation of Fc gamma RI or Fc epsilon RI. *J. Immunol.* **166:**4705–4712.

Okayama, Y., Hagaman, D. D., Woolhiser, M., and Metcalfe, D. D. (2001b). Further characterization of FcgammaRII and FcgammaRII expression by cultured human mast cells. *Int. Arch. Allergy Immunol.* **124:**155–157.

Okayama, Y., Kirshenbaum, A. S., and Metcalfe, D. D. (2000). Expression of a functional high-affinity IgG receptor, Fc gamma RI, on human mast cells: Up-regulation by IFN-gamma. *J. Immunol.* **164:**4332–4339.

Palucka, A. K. (2000). Dengue virus and dendritic cells. *Nature Med.* **6:**748–749.

Passlick, B., Flieger, D., and Ziegler-Heitbrock, H. W. (1989). Identification and characterization of a novel monocyte subpopulation in human peripheral blood. *Blood* **74:**2527–2534.

Patel, M., Yanagishita, M., Roderiquez, G., Bou-Habib, D. C., Oravecz, T., Hascall, V. C., and Norcross, M. A. (1993). Cell-surface heparan sulfate proteoglycan mediates HIV-1 infection of T-cell lines. *AIDS Res. Hum. Retrovir.* **9:**167–174.

Pavri, K., Swe, T., Ramamoorthy, C. L., and Chodankar, V. P. (1979). Immunoglobulin E in dengue haemorrhagic fever (DHF) cases. *Trans. R. Soc. Trop. Med. Hyg.* **73:**451–452.

Pavri, K. M., and Prasad, S. R. (1980). T suppressor cells: Role in dengue hemorrhagic fever and dengue shock syndrome. *Rev. Infect. Dis.* **2:**142–146.

Peerschke, E. I., and Ghebrehiwet, B. (1987). Human blood platelets possess specific binding sites for C1q. *J. Immunol.* **138:**1537–1541.

Peerschke, E. I., and Ghebrehiwet, B. (1998). Platelet receptors for the complement component C1q: Implications for hemostasis and thrombosis. *Immunobiology* **199:**239–249.

Peiris, J. S., and Porterfield, J. S. (1979). Antibody-mediated enhancement of Flavivirus replication in macrophage-like cell lines. *Nature* **282:**509–511.

Perussia, B., Dayton, E. T., Lazarus, R., Fanning, V., and Trinchieri, G. (1983). Immune interferon induces the receptor for monomeric IgG1 on human monocytic and myeloid cells. *J. Exp. Med.* **158:**1092–1113.

Pesci, A., Majori, M., Piccoli, M. L., Casalini, A., Curti, A., Franchini, D., and Gabrielli, M. (1996). Mast cells in bronchiolitis obliterans organizing pneumonia: Mast cell hyperplasia and evidence for extracellular release of tryptase. *Chest* **110:**383–391.

Phanichyakarn, P., Israngkura, P. B., Krisarin, C., Pongpanich, B., Dhanamitta, S., and Valyasevi, A. (1977a). Studies on dengue hemorrhagic fever: IV. Fluorescence staining of the immune complexes on platelets. *J. Med. Assoc. Thai.* **60:**307–311.

Phanichyakarn, P., Pongpanich, B., Israngkura, P. B., Dhanamitta, S., and Valyasevi, A. (1977b). Studies on Dengue hemorrhagic fever: III. Serum complement (C3) and platelet studies. *J. Med. Assoc. Thai.* **60:**301–306.

Phillpotts, R. J., Stephenson, J. R., and Porterfield, J. S. (1985). Antibody-dependent enhancement of tick-borne encephalitis virus infectivity. *J. Gen. Virol.* **66:**1831–1837.

Pincus, S., Mason, P. W., Konishi, E., Fonseca, B. A. L., Shope, R. E., Rice, C. M., and Paoletti, E. (1992). Recombinant vaccinia virus producing the prM and E proteins of yellow fever virus protects mice from lethal yellow fever encephalitis. *Virology* **187:**290–297.

Pletnev, S. V., Zhang, W., Mukhopadhyay, S., Fisher, B. R., Hernandez, R., Brown, D. T., Baker, T. S., Rossmann, M. G., and Kuhn, R. J. (2001). Locations of carbohydrate sites on alphavirus glycoproteins show that E1 forms an icosahedral scaffold. *Cell* **105:**127–136.

Ploegh, H. L. (1998). Viral strategies of immune evasion. *Science* **280:**248–253.

Pulimood, A. B., Mathan, M. M., and Mathan, V. I. (1998). Quantitative and ultrastructural analysis of rectal mucosal mast cells in acute infectious diarrhea. *Dig. Dis. Sci.* **43:**2111–2116.

Ramos, C., Sanchez, G., Pando, R. H., Baquera, J., Hernandez, D., Mota, J., Ramos, J., Flores, A., and Llausas, E. (1998). Dengue virus in the brain of a fatal case of hemorrhagic dengue fever. *J. Neurovirol.* **4:**465–468.

Ramos-Castaneda, J., Imbert, J. L., Barron, B. L., and Ramos, C. (1997). A 65-kDa trypsin-sensible membrane cell protein as a possible receptor for dengue virus in cultured neuroblastoma cells. *J. Neurovirol.* **3:**435–440.

Randolph, V. B., Winkler, G., and Stollar, V. (1990). Acidotropic amines inhibit proteolytic processing of flavivirus prM protein. *Virology* **174:**450–458.

Ravetch, J. V. (1994). Fc receptors: Rubor redux. *Cell* **78:**553–560.

Rey, F. A., Heinz, F. X., Mandl, C., Kunz, C., and Harrison, S. C. (1995). The envelope glycoprotein from tick-borne encephalitis virus at 2A resolution. *Nature* **375:**291–298.

Rice, C. M. (1996). *In* "Fields Virology" (B. N. Fields, D. M. Knipe, and P. M. Howley, eds.), 3rd Ed., pp. 931–959. Lippincott-Raven, Philadelphia.

Rico-Hesse, R., Harrison, L. M., Salas, R. A., Tovar, D., Nisalak, A., Ramos, C., Boshell, J., de Mesa, M. T., Nogueira, R. M., and da Rosa, A. T. (1997). Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. *Virology* **230:**244–251.

Rigau-Perez, J. G., Clark, G. G., Gubler, D. J., Reiter, P., Sanders, E. J., and Vorndam, A. V. (1998). Dengue and dengue haemorrhagic fever. *Lancet* **352:**971–977.

Rink, L., and Kirchner, H. (1996). Recent progress in the tumor necrosis factor–alpha field. *Int. Arch. Allergy Immunol.* **111:**199–209.

Roebuck, K. A., and Finnegan, A. (1999). Regulation of intercellular adhesion molecule-1 (CD54) gene expression. *J. Leukocyte Biol.* **66:**876–888.

Rosen, L., and Khin, M. M. (1989). Recovery of virus from the liver of children with fatal dengue: Reflections on the pathogenesis of the disease and its possible analogy with that of yellow fever. *Res. Virol.* **140:**351–360.

Rothman, A. L., and Ennis, F. A. (1999). Immunopathogenesis of dengue hemorrhagic fever. *Virology* **257:**1–6.

Rothwell, S. W., Putnak, R., and La Russa, V. F. (1996). Dengue-2 virus infection of human bone marrow: Characterization of dengue-2 antigen-positive stromal cells. *Am. J. Trop. Med. Hyg.* **54:**503–510.

Ruangjirachuporn, W., Boonpucknavig, S., and Nimmanitya, S. (1979). Circulating immune complexes in serum from patients with dengue haemorrhagic fever. *Clin. Exp. Immunol.* **36:**46–53.

Rue, C. A., and Ryan, P. (2002). Characterization of pseudorabies virus glycoprotein C attachment to heparan sulfate proteoglycans. *J. Gen. Virol.* **83:**301–309.

Russell, P. K., Intavivat, A., and Kanchanapilant, S. (1969). Anti-dengue immunoglobulins and serum beta 1 c/a globulin levels in dengue shock syndrome. *J. Immunol.* **102:**412–420.

Saadi, S., Holzknecht, R. A., Patte, C. P., and Platt, J. L. (2000). Endothelial cell activation by pore-forming structures: Pivotal role for interleukin-1alpha. *Circulation* **101:**1867–1873.

Saadi, S., Holzknecht, R. A., Patte, C. P., Stern, D. M., and Platt, J. L. (1995). Complement-mediated regulation of tissue factor activity in endothelium. *J. Exp. Med.* **182:**1807–1814.

Saadi, S., and Platt, J. L. (1995). Transient perturbation of endothelial integrity induced by natural antibodies and complement. *J. Exp. Med.* **181:**21–31.

Sa-Carvalho, D., Rieder, E., Baxt, B., Rodarte, R., Tanuri, A., and Mason, P. W. (1997). Tissue culture adaptation of foot-and-mouth disease virus selects viruses that bind to heparin and are attenuated in cattle. *J. Virol.* **71:**5115–5123.

Sahaphong, S., Riengrojpitak, S., Bhamarapravati, N., and Chirachariyavej, T. (1980). Electron microscopic study of the vascular endothelial cell in dengue haemorrhagic fever. *SE. Asian J. Trop. Med. Publ. Hlth.* **11:**194–204.

Saito, H., Miura, K., Takahashi, G., Ebisawa, M., Matsumoto, K., Shichijo, M., Onda, T., Iikura, Y., Yanagihara, Y., and Ra, C. (1995). Development of tryptase-positive KU812 cells cultured in the presence of Steel factor. *Int. Arch. Allergy Immunol.* **107:**330–332.

Salas-Benito, J., and del Angel, R. (1997). Identification of two surface proteins from C6/36 cells that bind dengue type 4 virus. *J. Virol.* **71:**7246–7252.

Scheppler, J. A., Nicholson, J. K., Swan, D. C., Ahmed-Ansari, A., and McDougal, J. S. (1989). Down-modulation of MHC-I in a CD4+ T cell line, CEM-E5, after HIV-1 infection. *J. Immunol.* **143:**2858–2866.

Schlesinger, J. J., and Brandriss, M. W. (1981a). Antibody-mediated infection of macrophages and macrophage-like cell lines with 17D-yellow fever virus. *J. Med. Virol.* **8:**103–117.

Schlesinger, J. J., and Brandriss, M. W. (1981b). Growth of 17D yellow fever virus in a macrophage-like cell line, U937: Role of Fc and viral receptors in antibody-mediated infection. *J. Immunol.* **127:**659–665.

Schlesinger, J. J., and Chapman, S. E. (1999). Influence of the human high-affinity IgG receptor FcgammaRI (CD64) on residual infectivity of neutralized dengue virus. *Virology* **260:**84–88.

Schnittler, H.-J., Wilke, A., Gress, T., Suttorp, N., and Drenckhahn, D. (1990). Role of actin and myosin in the control of paracellular permeability in pig, cat and human vascular endothelium. *J. Physiol.* **431:**379–401.

Schwartz, L. B., and Austen, K. F. (1984). Structure and function of the chemical mediators of mast cells. *Prog. Allergy* **34:**271–321.

Scott, R. M., Nisalak, A., Cheamudom, U., Seridhoranakul, S., and Nimmannitya, S. (1978). A preliminary report on the isolation of viruses from the platelets and leukocytes of dengue patients. *Asian J. Infect. Dis.* **2:**95–97.

Scott, R. M., Nisalak, A., Cheamudon, U., Seridhoranakul, S., and Nimmannitya, S. (1980). Isolation of dengue viruses from peripheral blood leukocytes of patients with hemorrhagic fever. *J. Infect. Dis.* **141:**1–6.

Selvan, R. S., Kapadia, H. B., and Platt, J. L. (1998). Complement-induced expression of chemokine genes in endothelium: Regulation by IL-1-dependent and -independent mechanisms. *J. Immunol.* **161:**4388–4395.

Selvaraj, P., Rosse, W. F., Silber, R., and Springer, T. (1988). The major Fc receptor in blood has a phosphatidylinositol anchor and is deficient in paroxysmal nocturnal haemoglobinuria. *Nature* **333:**565–567.

Selye, H. (1966). Mast cells and necrosis. *Science* **152:**1371–1372.

Selye, H., Somogyi, A., and Mecs, I. (1968). Influence of mast cells and vasoconstrictors upon various acute connective-tissue reactions. *Angiologica* **5:**172–185.

Seya, T., Turner, J. R., and Atkinson, J. P. (1986). Purification and characterization of a membrane protein (gp45–70) that is a cofactor for cleavage of C3b and C4b. *J. Exp. Med.* **163:**837–855.

Shaio, M.-F., Chang, F.-Y., and Hou, S.-C. (1992). Complement pathway activity in serum from patients with classical dengue fever. *Trans. Roy. Soc. Trop. Med. Hyg.* **86:**672–675.

Shanley, T. P., Warner, R. L., and Ward, P. A. (1995). The role of cytokines and adhesion molecules in the development of inflammatory injury. *Mol. Med. Today* **1:**40–45.

Shapiro, D., Brandt, W. E., and Russell, P. K. (1972). Change involving a viral membrane glycoprotein during morphogenesis of group B arboviruses. *Virology* **50:**906–911.

Shen, J., Devery, J. M., and King, N. J. (1995a). Adherence status regulates the primary cellular activation responses to the flavivirus West Nile. *Immunology* **84:**254–264.

Shen, J., Devery, J. M., and King, N. J. (1995b). Early induction of interferon-independent virus-specific ICAM-1 (CD54) expression by flavivirus in quiescent but not proliferating fibroblasts: Implications for virus-host interactions. *Virology* **208:**437–449.

Shen, J., To, S. S., Schrieber, L., and King, N. J. (1997). Early E-selectin, VCAM-1, ICAM-1, and late major histocompatibility complex antigen induction on human endothelial cells by flavivirus and comodulation of adhesion molecule expression by immune cytokines. *J. Virol.* **71:**9323–9332.

Shukla, D., Liu, J., Blaiklock, P., Shworak, N. W., Bai, X., Esko, J. D., Cohen, G. H., Eisenberg, R. J., Rosenberg, R. D., and Spear, P. G. (1999). A novel role for 3-O-sulfated heparan sulfate in herpes simplex virus 1 entry. *Cell* **99:**13–22.

Shukla, D., and Spear, P. G. (2001). Herpesviruses and heparan sulfate: An intimate relationship in aid of viral entry. *J. Clin. Invest.* **108:**503–510.

Simmons, D., and Seed, B. (1988). The Fcγ receptor of natural killer cells is a phospholipid-linked membrane protein. *Nature* **333:**568–570.

Sittisombut, N., Maneekarn, N., Kanjanahaluethai, A., Kasinrerk, W., Viputtikul, K., and Supawadee, J. (1995). Lack of augmenting effect of interferon-gamma on dengue virus multiplication in human peripheral blood monocytes. *J. Med. Virol.* **45:**43–49.

Smith, C. W., Marlin, S. D., Rothlein, R., Toman, C., and Anderson, D. C. (1989). Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. *J. Clin. Invest.* **83:**2008–2017.

Sobel, A. T., Bokisch, V. A., and Muller-Eberhard, H. J. (1975). C1q deviation test for the detection of immune complexes, aggregates of IgG, and bacterial products in human serum. *J. Exp. Med.* **142:**139–150.

Sparer, T. E., and Gooding, L. R. (1998). Suppression of MHC class I antigen presentation by human adenoviruses. *Curr. Top. Microbiol. Immunol.* **232:**135–147.

Sperr, W. R., Bankl, H. C., Mundigler, G., Klappacher, G., Grosschmidt, K., Agis, H., Simon, P., Laufer, P., Imhof, M., and Radaszkiewicz, T. (1994). The human cardiac mast cell: Localization, isolation, phenotype, and functional characterization. *Blood* **84:**3876–3884.

Springer, T. A. (1995). Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. *Annu. Rev. Physiol.* **57:**827–872.

Srichaikul, T., Nimmannitya, S., Sripaisarn, T., Kamolsilpa, M., and Pulgate, C. (1989). Platelet function during the acute phase of dengue hemorrhagic fever. *SE. Asian J. Trop. Med. Publ. Hlth.* **20:**19–25.

Steele, K. E., Linn, M. J., Schoepp, R. J., Komar, N., Geisbert, T. W., Manduca, R. M., Calle, P. P., Raphael, B. L., Clippinger, T. L., Larsen, T., Smith, J., Lanciotti, R. S., Panella, N. A., and McNamara, T. S. (2000). Pathology of fatal West Nile virus infections in native and exotic birds during the 1999 outbreak in New York City, New York. *Vet. Pathol.* **37:**208–224.

Stiasny, K., Allison, S. L., Marchler-Bauer, A., Kunz, C., and Heinz, F. X. (1996). Structural requirements for low-pH–induced rearrangements in the envelope glycoprotein of tick-borne encephalitis virus. *J. Virol.* **70:**8142–8147.

Strauss, J. H., and Strauss, E. G. (2001). Virus evolution: How does an enveloped virus make a regular structure? *Cell* **105:**5–8.

Studahl, M., Rosengren, L., Gunther, G., and Hagberg, L. (2000). Difference in pathogenesis between herpes simplex virus type 1 encephalitis and tick-borne encephalitis demonstrated by means of cerebrospinal fluid markers of glial and neuronal destruction. *J. Neurol.* **247:**636–642.

Sung, J. S., Diwan, A. R., Falkler, W. A. J., Yang, H. Y., and Halstead, S. B. (1975). Dengue carrier culture and antigen production in human lymphoblastoid lines. *Intervirol.* **5:**137–149.

Suzuki, T., Ogata, A., Tashiro, K., Nagashima, K., Tamura, M., Yasui, K., and Nishihira, J. (2000). Japanese encephalitis virus up-regulates expression of macrophage migration inhibitory factor (MIF) mRNA in the mouse brain. *Biochim. Biophys. Acta* **1517:**100–106.

Suzumura, A., Lavi, E., Weiss, S. R., and Silberberg, D. H. (1986). Coronavirus infection induces H-2 antigen expression on oligodendrocytes and astrocytes. *Science* **232:**991–993.

Szabo, C., and Billiar, T. R. (1999). Novel roles of nitric oxide in hemorrhagic shock. *Shock* **12:**1–9.

Takasaki, T., Takada, K., and Kurane, I. (2001). Electron microscopic study of persistent dengue virus infection: analysis using a cell line persistently infected with dengue-2 virus. *Intervirol.* **44:**48–54.

Takeda, A., Robinson, J. E., Ho, D. D., Debouck, C., Haigwood, N. L., and Ennis, F. A. (1992). Distinction of human immunodeficiency virus type 1 neutralization and infection enhancement by human monoclonal antibodies to glycoprotein 120. *J. Clin. Invest.* **89:**1952–1957.

Takeda, A., Sweet, R. W., and Ennis, F. A. (1990). Two receptors are required for antibody-dependent enhancement of human immunodeficiency virus type 1 infection: CD4 and Fc gamma R. *J. Virol.* **64:**5605–5610.

Tamura, M., Webster, R. G., and Ennis, F. A. (1993). Neutralization and infection-enhancement epitopes of influenza A virus hemagglutinin. *J. Immunol.* **151:**1731–1738.

Tartaglia, L. A., and Goeddel, D. V. (1992). Two TNF receptors. *Immunol. Today* **13:**151–153.

Tassaneetrithep, B., Burgess, T. H., Granelli-Piperno, A., Trumpfheller, C., Finke, J., Sun, W., Eller, M. A., Pattanapanyasat, K., Sarasombath, S., Birx, D. L., Steinman, R. M., Schlesinger, S., and Marovich, M. A. (2003). DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. *J. Exp. Med.* **197:**823–829.

Taweechaisupapong, S., Sriurairatana, S., Angsubhakorn, S., Yoksan, S., and Bhamarapravati, N. (1996a). In vivo and in vitro studies on the morphological change in the monkey epidermal Langerhans cells following exposure to dengue 2 (16681) virus. *SE. Asian J. Trop. Med. Publ. Hlth.* **27:**664–672.

Taweechaisupapong, S., Sriurairatana, S., Angsubhakorn, S., Yoksan, S., Khin, M. M., Sahaphong, S., and Bhamarapravati, N. (1996b). Langerhans cell density and serological changes following intradermal immunisation of mice with dengue 2 virus. *J. Med. Microbiol.* **45:**138–145.

Thein, S., Aung, M. M., Shwe, T. N., Aye, M., Zaw, A., Aye, K., Aye, K. M., and Aaskov, J. (1997). Risk factors in dengue shock syndrome. *Am. J. Trop. Med. Hyg.* **56:**566–572.

Theofilopoulos, A. N., Brandt, W. E., Russell, P. K., and Dixon, F. T. (1976). Replication of dengue-2 virus in cultured human lymphoblastoid cells and subpopulations of human peripheral leukocytes. *J. Immunol.* **117:**953–961.

Timares, L., Takashima, A., and Johnston, S. A. (1998). Quantitative analysis of the immunopotency of genetically transfected dendritic cells. *Proc. Natl. Acad. Sci. USA.* **95:**13147–13152.

Tomita, M., Yamamoto, K., Kobashi, H., Ohmoto, M., and Tsuji, T. (1994). Immunohistochemical phenotyping of liver macrophages in normal and diseased human liver. *Hepatol.* **20:**317–325.

Tracey, K. J., and Cerami, A. (1993). Tumor necrosis factor, other cytokines and disease. *Annu. Rev. Cell Biol.* **9:**317–343.

Trirawatanapong, T., Chandran, B., Putnak, R., and Padmanabhan, R. (1992). Mapping of a region of dengue virus type-2 glycoprotein required for binding by a neutralizing monoclonal antibody. *Gene* **116:**139–150.

Tuchinda, M., Dhorranintra, B., and Tuchinda, P. (1977). Histamine content in 24-hour urine in patients with dengue haemorrhagic fever. *SE. Asian J. Trop. Med. Publ. Hlth.* **8:**80–83.

Tuijnman, W. B., Van Wichen, D. F., and Schuurman, H. J. (1993). Tissue distribution of human IgG Fc receptors CD16, CD32 and CD64: an immunohistochemical study. *APMIS* **101:**319–329.

van Den Engel, N. K., Heidenthal, E., Vinke, A., Kolb, H., and Martin, S. (2000). Circulating forms of intercellular adhesion molecule (ICAM)-1 in mice lacking membranous ICAM-1. *Blood* **95:**1350–1355.

van der Most, R. G., Corver, J., and Strauss, J. H. (1999). Mutagenesis of the RGD motif in the yellow fever virus 17D envelope protein. *Virol.* **265:**83–95.

van de Winkel, J. G. J., and Anderson, C. L. (1991). Biology of human immunoglobulin G Fc receptors. *J. Leuk. Biol.* **49:**511–524.

van Epps, D. E., and Chenoweth, D. E. (1984). Analysis of the binding of fluorescent C5a and C3a to human peripheral blood leukocytes. *J. Immunol.* **132:**2862–2867.

Vassalli, P. (1992). The pathophysiology of tumor necrosis factors. *Annu. Rev. Immunol.* **10:**411–452.

Vitarana, T., de Silva, H., Withana, N., and Gunasekera, C. (1991). Elevated tumour necrosis factor in dengue fever and dengue haemorrhagic fever. *Ceylon Med. J.* **36:**63–65.

Wang, S., He, R., and Anderson, R. (1999). PrM- and cell-binding domains of the dengue virus E protein. *J. Virol.* **73:**2547–2551.

Wang, S., He, R., Patarapotikul, J., Innis, B. L., and Anderson, R. (1995). Antibody-enhanced binding of dengue-2 virus to human platelets. *Virol.* **213:**254–257.

Wang, S., Zang, L., Feng, M., Liang, Z., Zheng, S., Zhang, L., Jiang, Z., and Chen, D. (1997). Transmission electron microscopic study of the hemorrhagic spots in

patients with epidemic hemorrhagic fever in the early stage. *Ultrastruct. Pathol.* **21:**281–287.

Wedi, B., Lewrick, H., Butterfield, J. H., and Kapp, A. (1996). Human HMC-1 mast cells exclusively express the Fc gamma RII subtype of IgG receptor. *Arch. Dermatol. Res.* **289:**21–27.

Weiner, L. P., Cole, G. A., and Nathanson, N. (1970). Experimental encephalitis following peripheral inoculation of West Nile virus in mice of different ages. *J. Hyg. (Lond.)* **68:**435–446.

Weiss, H. J., and Halstead, S. B. (1965). Studies of hemostasis in Thai hemorrhagic fever. *J. Pediat.* **66:**918–926.

Wengler, G. (1989). Cell-associated West Nile flavivirus is covered with E+pre-M protein heterodimers which are destroyed and reorganized by proteolytic cleavage during virus release. *J. Virol.* **63:**2521–2526.

Winkelstein, A., and Kiss, J. E. (1997). Immunohematologic disorders. *JAMA* **278:**1982–1992.

Wu, S. J., Grouard-Vogel, G., Sun, W., Mascola, J. R., Brachtel, E., Putvatana, R., Louder, M. K., Filgueira, L., Marovich, M. A., Wong, H. K., Blauvelt, A., Murphy, G. S., Robb, M. L., Innes, B. L., Birx, D. L., Hayes, C. G., and Frankel, S. S. (2000). Human skin Langerhans cells are targets of dengue virus infection. *Nature Med.* **6:**816–820.

Yadav, M., Kamath, K. R., Iyngkaran, N., and Sinniah, M. (1991). Dengue haemorrhagic fever and dengue shock syndrome: Are they tumour necrosis factor-mediated disorders? *FEMS Microbiol. Immunol.* **89:**45–50.

Yang, K. D., Yeh, W. T., Yang, M. Y., Chen, R. F., and Shaio, M. F. (2001). Antibody-dependent enhancement of heterotypic dengue infections involved in suppression of IFN-gamma production. *J. Med. Virol.* **63:**150–157.

Zaki, S. R., Shieh, W. J., Greer, P. W., Goldsmith, C. S., Ferebee, T., Katshitshi, J., Tshioko, F. K., Bwaka, M. A., Swanepoel, R., Calain, P., Khan, A. S., Lloyd, E., Rollin, P. E., Ksiazek, T. G., and Peters, C. J. (1999). A novel immunohistochemical assay for the detection of Ebola virus in skin: Implications for diagnosis, spread, and surveillance of Ebola hemorrhagic fever. *J. Infect. Dis.* **179**(Suppl. 1):S36–S47.

Zhu, X., Meng, G., Dickinson, B. L., Li, X., Mizoguchi, E., Miao, L., Wang, Y., Robert, C., Wu, B., Smith, P. D., Lencer, W. I., and Blumberg, R. S. (2001). MHC class I–related neonatal Fc receptor for IgG is functionally expressed in monocytes, intestinal macrophages, and dendritic cells. *J. Immunol.* **166:**3266–3276.

Zucker-Franklin, D., Seremetis, S., and Zheng, Z. Y. (1990). Internalization of human immunodeficiency virus type 1 and other retroviruses by megakaryocytes and platelets. *Blood* **75:**1920–1923.

Zwirner, J., Fayyazi, A., and Gotze, O. (1999). Expression of the anaphylatoxin C5a receptor in non-myeloid cells. *Mol. Immunol.* **36:**877–884.