Insights from direct studies on human dengue infections

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Biologic Functions of Human Antibodies

A close look at dengue viremia in humans resulted in a discovery that has rocked the dengue world. In PNAS, Raut et al. (1) cleverly manipulated viruses recovered from the blood of acute dengue virus type 1 (DENV1) infections and show that this virus differs structurally from DENVs grown in vitro. DENV1, -2, and -3 grown in cell culture consistently were poorly neutralized by polyclonal human monotypic DENV antibodies but strongly neutralized by human DENV cross-reactive antibodies (1). By contrast, DENV1 produced in vivo was neutralized strongly by homotypic antibodies but hardly at all by heterotypic DENV antibodies. For generations, researchers have measured neutralizing antibodies in sera from DENV-infected or vaccinated humans using DENV grown in cell cultures. Classic methods of measuring dengue-neutralizing antibodies to stratify dengue protection are now in serious jeopardy. We are now in the era of the human dengue challenge model. Attenuated DENV vaccine viruses and less well attenuated DENVs are being administered to susceptible adult volunteers (2). Challenge models could provide a source of primary DENV2, -3, and -4 infection to yield the samples needed to extend the pilot DENV1 studies reported here.

Careful study of dengue-infected humans is not new. In experiments at the dawn of the 20th century, mosquito transmission and dengue viremia in humans were demonstrated by feeding *Aedes aegypti* on acute-phase blood. Infected mosquitoes, as well as acute-phase blood from infected volunteers inoculated into susceptibles, demonstrated the infectious nature of the organism and, as a dividend, generated dengue fever cases for clinical study (3–9). Neutralization of DENVs by antibodies was first accomplished by mixing viremic human blood with serum from a convalescent patient and inoculating the mixture into a susceptible human (9). Subsequently, DENVs were adapted to grow in infant mouse brain, giving rise to a virus recovery system and simple test for neutralizing antibodies (10). More conveniently, DENVs were found to grow in cell culture. It became possible to count plaques formed by DENVs grown on cell culture monolayers to generate a gold-standard plaque reduction neutralizing antibody test (11).

Later during the 20th century, the four DENVs expanded geographically from endemic foci in Southeast Asia to produce a pandemic involving nearly the entire tropical and subtropical world, each year generating millions of infections and illnesses, mild and severe (12). This immense disease burden, coupled with the failure of vector control, stimulated a major program of dengue vaccine development. Throughout this process, two tests have been relied on to estimate efficacy: live-virus challenge of immunized animals, with emphasis on subhuman primates; and in vitro measurement of antibodies that neutralized cell culture-grown DENV. To the surprise of many, a large phase 3 clinical trial of a tetravalent live-attenuated dengue vaccine (Dengvaxia) failed to protect many of those who were vaccinated, despite ample production of neutralizing antibodies to all four DENVs (13).

Two of the phenomena described by Raut et al. may help us understand the failure to predict vaccine success, as well as solve other dengue mysteries. First, DENV1 grown in humans in vivo is consistently mature, while cell culture-grown DENVs are predominantly immature (1). The high frequency of tetravalent neutralization of immature DENV in phase 1 and 2 studies led to the widespread expectation that the vaccine would be protective. But breakthrough dengue infections of vaccinated subjects were common (13). This led to a spirited debate to explain why neutralizing antibodies did not correlate with protection. It was speculated, for example, that antigenic distance between the DENV in vaccine and the wild-type DENVs circulating during the phase 3 trial might contribute to protection failure (14). However, careful study of phase 3 sera found that the four cell culture-grown DENVs were neutralized predominantly by cross-reactive antibodies, with scant evidence of strongly neutralizing DENV-type–specific quaternary epitope antibodies in circulation (15). Another pathogenic DENV–antibody interaction that could be impacted by

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virion structure is the ability of maternal IgG DENV antibodies to enhance infant DENV infections when degraded naturally to enhancing concentrations (16). This phenomenon has been documented only for mothers who had experienced two or more DENV infections—never after a single dengue infection (17). In mouse models, monoclonal and polyclonal monotypic DENV antibodies regularly enhance DENV infections, producing vascular permeability disease and death (18). Something is wrong! Is it possible that mature dengue virions are not enhanced in vivo by the cross-reactive antibodies that are found in primary dengue infections? As noted by Raut et al. (1), human mature virions are poorly neutralized by cross-reactive antibodies.

Second, DENV1 grown in cell culture exhibits a low infectivity ratio compared with DENV1 produced in humans (1). How is the poorly infectious immature DENV in vaccines handled by the immune system compared with mature DENV? Is Vero cell-produced DENV processed to form "normal" quaternary epitope antibodies? Might viral immaturity contribute to the interference phenomenon long suspected to hamper production of DENV-type–specific neutralizing antibodies? These simple hypotheses have not yet been subjected to experimental scrutiny.

Cellular Pathogenesis of Severe Dengue

A final plea is to direct study at identifying sites of cellular DENV infections in humans. DENV antibodies perform two biological functions: to neutralize and enhance infection. We know that antibodies from natural DENV infections and those raised by Dengvaxia are capable of enhancing the severity of primary dengue infections (19, 20). As described many times, most human antibodies derived from primary or secondary dengue infections are capable of antibody-dependent enhancement (ADE) in vitro (21). Why is the clinical phenomenon of ADE relatively rare? How does ADE really work? Just as we have learned that DENVs produced in humans or cell cultures are structurally different, it is crucial to the biology of ADE to irrefutably identify dengue-infected target cells. Almost all recent studies on dengue ADE have used continuous cell lines, and virtually no one has attempted to quantitate ADE using the immunosuppressive capacity of infectious immune complexes (22). It should be recognized that studies on a non-DENV system resulted in the discovery of a remarkable phenomenon: intrinsic ADE (23). Intrinsic ADE in Ross River virus was extended to DENV using THP-1 cells (24), but then it was discovered that DENV–immune complex suppression of intracellular antiviral defenses differed between primary human monocytes and macrophages (25).

The key to designing ADE studies is identified in the Raut et al. report (1): "the precise cells that produce DENVs in people have not been defined well". Cultures of primary human monocytes from flavivirus-susceptible donors have been used to study ADE, but these are surrogates (26). The vast majority of Fc-receptor–bearing target cells that support DENV infection reside in tissues. What are the exact genotypic and phenotypic markers of the principal target cells supporting dengue infection? How do real target cells control ADE outcomes? As the organizer of an autopsy study on 13 children who died of shock syndrome (27), an endeavor that consumed 10 y, it is disappointing to acknowledge that target cells infected by DENVs have not yet been adequately identified. The scientific community must not accept the unchallenged belief that continuous Fc-receptor–bearing cell lines can be substituted for authentic human target cells to measure or study ADE. When we succeed in identifying in vivo dengue target cells, we will need to learn how to generate and work with these cells in experimental systems. How might that discovery translate to an understanding of the infection dynamics of the real human target cells that produce a virus that kills people?

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Yes, DENVs are killers. Dengue nonstructural protein 1 (NS1) has direct toxic properties, making dengue shock syndrome a viral analog of bacterial toxic shock syndrome. DENV NS1 produces endothelial hyperpermeability in vitro and vascular leak in vivo by destruction of the gel-like endothelial glycocalyx layer (EGL), and can do so in the absence of DENV infection. NS1 exacerbates an otherwise sublethal DENV infection. Cytokines may have no pathological role in vascular permeability, as a mouse model shows that circulation of NS1 alone leads to vascular permeability accompanied by significant increases in circulating levels of inflammatory cytokines (28). DENV NS1 disrupts the EGL in vitro through an activation of endothelial sialidases and the cathepsin L/heparanase pathway. Glycocalyx components, such as heparan sulfate and chondroitin sulfate, circulate at higher levels in the sera of DENV-infected patients as compared with healthy controls (28). These findings have profound implications for moderation of acute illness by use of agents that interrupt the EGL destruction cascade or prevent NS1-mediated dengue vascular permeability, possibly with a vaccine.

Meanwhile, a mystery prevails in understanding the pathophysiology of the NS1 dengue toxic shock syndrome. In animal models, endothelial cells are rapidly damaged by DENV NS1. But in humans, severe vascular permeability is a rare event, given the ubiquitous circulation of DENV NS1. Furthermore, vascular decompensation is very closely linked to defervescence, a time when circulating levels of NS1 are low. Defervescence in viral diseases usually signals the termination of intracellular infection by cell-mediated immunity. Could the destruction of DENV-infected target cells lead to the rapid release of NS1 and then to endothelial cell destruction? Many challenges remain; they may benefit from continuing to focus carefully and critically and directly on dengue-infected humans.

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