COMMENTARY

Original antigenic sin in dengue revisited

Simona Zompi and Eva Harris[1]

Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, CA 94720-3370

The four dengue virus serotypes (DENV-1 to -4) cause the most important arthropod-borne viral disease of humans, with ~100 million cases each year and over 3 billion people at risk for infection (1). The immune response to DENV infection is complex, because it can be either protective or pathogenic. The "original antigenic sin" in secondary (2°) DENV infections is defined as the dominance of cross-reactive antibodies or T-cell responses to a first infecting DENV serotype (the "original antigen") over the current infecting serotype. In acute DENV infections, cross-reactive T-cell responses have been associated with more severe disease, consigning cross-reactive T-cell responses to a pathogenic role (2, 3). In this issue of PNAS, Weiskopf et al. (4) challenge the idea that cross-reactive T-cell responses are only associated with pathogenesis by demonstrating that although $CD8^+$ T-cell responses were indeed skewed toward the first DENV infection, this did not result in impaired responses, either qualitatively or quantitatively. Furthermore, they found that higher magnitude T-cell responses were associated with HLA alleles that have been linked to reduced susceptibility to severe dengue. Thus, these results suggest that human cross-reactive T-cell responses can be associated with a robust and multifunctional response that can induce protection, as has been shown in dengue mouse models (5–7).

Dengue epidemiology and immune response

Dengue is endemic throughout the world's subtropical and tropical regions, especially in Asia and Latin America, and it is considered an emerging infectious disease threat in the United States and a category A pathogen. Increased urbanization, globalization, and travel, as well as climate change, all contribute to its uncontrolled expansion (8). The acute febrile illness dengue fever (DF) can progress to a potentially life-threatening vascular leakage syndrome, known as dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), the latter characterized by hypotension and circulatory failure, most often affecting children (9). There are ~500,000 hospitalizations attributable to dengue each year, with case fatality rates as high as 10–20% without appropriate treatment (9). Dengue constitutes a substantial economic burden in endemic countries (10), which are mostly low- and middle-income countries. No specific treatment exists, and an incomplete understanding of the immune response has hindered vaccine development.

Primary (1°) infection with any of the four DENV serotypes is believed to confer lifelong protection to the homologous serotype; however, 2° infection with a different DENV serotype is the major risk factor for severe disease (11). This may be attributable to cross-reactive T cells (3) or to "antibody-dependent enhancement" (12), where cross-reactive anti-DENV antibodies facilitate entry of DENV into constant fragment receptor-bearing cells. However, the majority of 2° DENV infections are asymptomatic or result in only mild disease. The immune mechanisms underlying protection against a heterologous DENV infection are not well understood. The unexpected results of the first proof-of-concept dengue tetravalent live attenuated vaccine efficacy trial, in which the CYD23 vaccine failed to protect against DENV-2 (13), highlight the critical need to better understand the immune response to natural DENV infections and vaccine candidates and to identify robust correlates of protection, especially because in vitro neutralization titers did not correlate with vaccine efficacy. CYD23 is a chimeric vaccine containing the nonstructural proteins from yellow fever virus and the structural proteins (prM/M and E) from DENV. Because most T-cell epitopes are found in the nonstructural proteins, this vaccine does not induce a DENV-specific $CD8^+$ T-cell response. Thus, it will be important to explore T-cell responses as possible correlates of protection.

Expansion of the DENV T cell epitope database

T-cell epitopes are ~10–20 aa in length, presented on the surface of antigen-presenting cells, that bind major histocompatibility complex (MHC) on the surface of T cells. One limitation of T-cell studies in human DENV infections has been the low number of characterized DENV T-cell epitopes. Weiskopf et al. (4) focused on the $CD8^+$ T-cell response, thus on MHC class I molecules, in Sri Lankan blood donors. They started by synthesizing a very large number of peptides (8,088) that bound the 27 most prominent MHC class I alleles (HLA-A and HLA-B) identified in the Sri Lankan population. By choosing this set of 27 alleles, the authors were able to match three of four MHC class I alleles in 90% of the donors (figure 1 in ref. 4). Among these, they identified 408 unique DENV epitopes, increasing by fivefold the number of DENV epitopes currently available in the Immune Epitope Database. These epitopes are publicly accessible to the research community and will be useful for future T-cell studies. Among these 408 epitopes, the authors were able to characterize only 25 immunodominant epitopes that account for 50% of the T-cell responses in the population studied, thus enabling future measurement of $CD8^+$ T-cell responses using small volumes of blood. This is particularly relevant for DENV infections, because many symptomatic and severe cases are reported in children, and pediatric samples are always available in limited amounts. In addition, the previous definition of immunodominant peptides could be skewed. Although several groups have reported immunodominant epitopes in NS5, NS4B, and E (14, 15), most of the DENV T-cell epitopes studied thus far are localized in the NS3 protein (3). The work of Weiskopf et al. now provides a broader picture of the immunodominant $CD8^+$ T-cell epitope repertoire across the entire DENV genome (4).

A protective role for original antigenic sin

Several lines of evidence suggest that both $CD4^+$ and $CD8^+$ T cells help resolve DENV infection; for instance, serotype-specific $CD4^+$ and $CD8^+$ T-cell responses are observed in humans with 1° DENV infection (16), and DENV-specific human $CD4^+$ T cells specific for NS3 proliferate, produce IFN-γ, and can lyse target cells (17). However, altered T-cell

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[1]To whom correspondence should be addressed. E-mail: eharris@berkeley.edu.

responses during 2° infections with a different DENV serotype may contribute to the "cytokine storm" and immunopathogenesis of DHF/DSS. Several studies have shown that serotype cross-reactive T cells are preferentially activated during 2° infection and exhibit suboptimal degranulation and enhanced TNF-α and IFN-γ production (2). Another study found the ratio of TNF-α– to IFN-γ–producing cells was higher when DENV-specific $CD4^+$ T cells were stimulated ex vivo with antigens from the heterologous vs. homologous serotype (18). Increased TNF-α production by T cells could facilitate vascular leakage, as TNF-α has been detected more frequently and at higher levels in the serum of patients with DHF/DSS than with DF (19). Overall, studies in human DENV infections thus far have mostly focused on the potential role of T cells in pathogenesis and the association between HLA alleles and disease severity.

The sequence of infecting DENV serotypes can modulate disease severity (20). Defining the sequence of infecting DENV serotypes is difficult, except in the context of cohort studies, where individuals are followed longitudinally over many years and have well-characterized sequential DENV infections. In the absence of a well-characterized cohort, Weiskopf et al. (4) studied adult blood donors from the general population for which prior longitudinal samples were not available. They used Sri Lankan epidemiologic data and serological assays to infer the initial infecting DENV serotype. In Sri Lanka, DENV-2 has dominated epidemics throughout the years, with more recent introduction of DENV-3 and DENV-1 (21). Most adults in Sri Lanka are seropositive for DENV (i.e., they have previously experienced infection by at least one DENV serotype). Using serological results from both ELISA and neutralization assays, Weiskopf et al. (4) defined infection as either 1° or 2°. The most likely infecting serotypes were defined by the $CD8^+$ T-cell response measured by the IFNγ response in ex vivo ELISPOT assays against serotype-specific epitopes (table 2 in ref. 4). Based on epidemiological data, the authors hypothesized that most donors who experienced a 2° infection had undergone a 1° infection with DENV-2 followed by one of the other serotypes. Donors responding to serotype-specific DENV-2 epitopes had a ratio of specific to conserved (cross-reactive) responses skewed toward the serotype-specific DENV-2 epitopes, whereas donors responding to the other serotype-specific epitopes had a ratio of specific to conserved responses skewed toward the conserved epitopes (table 2 in ref. 4). These data corroborate the hypothesis of original antigenic sin (i.e., conserved, or cross-reactive, epitopes dominate the $CD8^+$ T-cell response in 2° DENV infections).

Weiskopf et al. (4) describe a thorough and comprehensive analysis of $CD8^+$ T-cell functions. Of note, these experiments were conducted directly ex vivo, in contrast to responses measured after in vitro stimulation for several days. Importantly, no difference in $CD8^+$ T-cell responses were noted in terms of magnitude, phenotype, CD107a expression (a marker of cytotoxicity), cytokine profile/polyfunctionality, and avidity in serotype-specific vs. conserved responses. Thus, in addition to its role in pathogenesis, "original antigenic sin" in human DENV infections has now been associated with T-cell functions characteristic of T-cell–mediated protection.

Another important feature of this paper is that the authors HLA-typed the same donors and associated HLA genotyping with T-cell function (4). Previous data have shown an association between HLA types and dengue disease susceptibility (22). Rather than the serotype-specific vs. conserved nature of the response, Weiskopf et al. (4) found that it is the magnitude of the T-cell response that is associated with HLA types that correlate with disease susceptibility (figure 4 in ref. 4), suggesting that a correlate of protection is not the type of epitope but rather the magnitude of the response to certain epitopes. The authors also show that a higher magnitude $CD8^+$ T-cell response is associated with greater multifunctionality, a characteristic of protective responses.

The experiments reported by Weiskopf et al. (4) were conducted in the general population, and sampling likely occurred several years after the last DENV infection. As such, these data are not necessarily in contradiction with previously published data showing a pathogenic role for T cells during acute DENV infections (2). A robust cross-reactive T-cell response could be protective in general but in specific cases, seen in the acute phase of certain 2° DENV infections with severe disease, cross-reactive T-cell responses might contribute to pathogenesis. Pathogenesis of acute 2° DENV infections is complex, and numerous factors influence how cross-reactive T cells behave during the acute phase of the infection, including pre-existing immunity, the time interval between DENV infections, host genetic factors, the sequence of serotypes, and viral load.

Conclusions

These results provide a refreshing view of the role of T cells in human DENV infection. Both T and B cells can play a protective or pathogenic role in dengue but more data are needed in human populations, and Weiskopf et al. (4) provide important evidence of the protective role of $CD8^+$ T cells through their studies of Sri Lankan blood donors. The next step will be to extend this work into clinical studies of individuals with defined dengue disease outcomes and defined serotype sequences of infection. In addition, although this study may be generalizable to Asian populations, a similar analysis in the Americas will be important as well. Overall, this work is particularly timely given the urgent need to identify correlates of protection of relevance to ongoing vaccine trials and development of new vaccine candidates.

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