

2013

# Immunopathogenesis versus protection in dengue virus infections

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## Citation/Publisher Attribution

Rothman, A. L., Medin, C. L., Friberg, H., & Currier, J. R. (December 2013). Immunopathogenesis versus protection in dengue virus infections. *Current Tropical Medicine Reports*, 1-9.

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## **Immunopathogenesis versus protection in dengue virus infections**

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## **Abstract**

Dengue viruses (DENV) are mosquito-borne viruses that cause significant morbidity. The existence of four serotypes of DENV with partial immunologic cross-reactivity creates the opportunity for individuals to experience multiple acute DENV infections over the course of their lifetimes. Research over the past several years has revealed complex interactions between DENV and the human innate and adaptive immune systems that can have either beneficial or detrimental influences on the outcome of infection. Further studies that seek to distinguish protective from pathological immune responses in the context of natural DENV infection as well as clinical trials of candidate DENV vaccines have an important place in efforts to control the global impact of this re-emerging viral disease.

Keywords: dengue, immunopathogenesis, vaccine, T lymphocyte, innate immunity

## Introduction

Two seminal observations have had a major and long-standing impact on progress in development of vaccines for prevention of infection with dengue virus (DENV). First, work done during World War II by Sabin and others demonstrated long-lasting resistance to reinfection with the same DENV serotype, in contrast to only short-term resistance to infection with heterologous serotypes [1]. Having established the generation of protective immunity, the path to development of an effective vaccine seemed straight-forward. Subsequently, Halstead and colleagues noted the association of dengue hemorrhagic fever (DHF) with secondary DENV infections [2], an association that was later convincingly demonstrated in prospective cohort studies [3-5]. The immune enhancement model of DHF pathogenesis, developed from these observations [6], has presented a cautionary note for vaccination against DENV. The 'holy grail' in DENV immunology has therefore been to achieve a sufficiently comprehensive base of knowledge to distinguish protective DENV-specific immunity from pathological DENV-specific immunity.

A brief summary is in order. DENV refers to a complex of four closely related flaviviruses, termed serotypes and designated DENV-1, DENV-2, DENV-3, and DENV-4 [7]. All four viruses are transmitted between humans and mosquitoes of the genus *Aedes*, demonstrate tropism for monocytes, macrophages, and dendritic cells, and cause similar clinical syndromes, including mild dengue, classical dengue fever (DF), and DHF. Although its name suggests otherwise, the principal feature of DHF is increased vascular permeability, which causes excessive plasma leakage manifesting as increased hematocrit (hemoconcentration) and effusions in pleural and peritoneal spaces and can result in life-threatening shock.

The focus of this review is recent virology and immunology research on DENV that has yielded a better, if ever more complex, picture of the contribution of the immune responses to the outcomes—

from both viral and host perspectives— of infection (Figure 1). As the field moves towards an improved understanding of the kinetics of infection and disease, and key pathways of innate and adaptive immunity and their interactions, potential implications for vaccine development and testing are also coming into sharper perspective.

### **Kinetics of dengue viral replication and immune responses in vivo**

Prospective longitudinal studies of individuals with acute dengue illness have revealed a highly dynamic interaction between the virus and the host immune response. A consistent finding is that peak viremia titers are coincident with the onset of fever and other classical dengue symptoms [8, 9]. Levels of DENV RNA in both plasma and peripheral blood leukocytes decline thereafter [10] and are <1% of their peak values by the time plasma leakage occurs (in patients with DHF). A significant positive correlation can be demonstrated between viremia titers and illness severity, but only when looking at peak values. Field studies involving more active blood sampling in dengue-endemic areas have extended these findings, showing significantly lower viremia titers in individuals with very mild dengue illness and in asymptomatic individuals [11]. At the same time, a substantial fraction of individuals with high peak viremia titers do not develop plasma leakage. Taken as a whole, these observations suggest that high viremia is necessary but not sufficient for the development of plasma leakage and may act as a trigger for other responses that directly lead to increased vascular permeability.

Activation of innate and adaptive immune mechanisms is also a consistent finding in patients with acute dengue illness. Based on the plasma levels of cytokines and soluble cell activation markers [12] as well as by gene expression analysis [13, 14] and flow cytometry [15] of circulating leukocytes, multiple pathways are activated. A somewhat counterintuitive finding is that immune responses that are associated with both the promotion of inflammation (e.g., IFN- $\gamma$  and TNF $\alpha$ ) and the inhibition of inflammation (e.g., IL-10) are activated in dengue. Studies have shown significant correlations among

these different measures and between immune activation and viremia, however, which complicate efforts to infer causality with regard to disease. In this regard, the kinetics of immune activation appears to be a critical consideration. Whereas peak viremia uniformly occurs early in illness, each of the different immune responses follows a distinct temporal pattern. Type I IFN levels, for example, peak early during illness, in parallel with viremia levels, whereas levels of IL-10 and VEGF peak later, closer in time to plasma leakage. In one study, the relative timing of peak viremia, IFN- $\gamma$ , and IL-10 was significantly different between patients with DF and those with DHF [16]. We have favored the interpretation that elevated levels of IL-10 represent a counter-regulatory response to the activation of pro-inflammatory pathways.

### **Effects of dengue virus infection on host cells- intersection of innate and adaptive responses**

While the association of severe disease with secondary DENV infection has focused attention on the role of adaptive immune responses, substantial progress has been made in understanding innate immunity in dengue and its interactions with adaptive immunity. In vitro studies have elucidated a variety of structural changes within the cell that are induced by DENV infection and affect (or are predicted to affect) the immune response to acute infection. Some of the most striking changes are an increase in ER-derived membranes, structural changes to mitochondria, and an abundance of vesicles containing viral particles [17, 18]. Several studies have shown that autophagosomes are induced by DENV infection and contribute to viral replication [19-23]. Many of the organelles affected by DENV infection also play a role in the cellular antiviral response. DENV triggers type I IFN production through the RIG-I signaling pathway [24, 25]. RIG-I and a recently identified molecule, STING (also known as MITA, ERIS, and TMEM173), interact with MAVS at the mitochondrial membrane to activate IRF3 and induce type I IFN production [26]. Autophagosomes deliver viral proteins to the antigen presentation pathway and also regulate Toll-like receptor activation [27].

The regulation of these innate immunity pathways by viruses such as DENV has also been an active area of research. The DENV replication complex is located in close association with cellular membranes, where the viral proteins and RNA appear to interact with essential host cell factors [28]. DENV has been shown to inhibit type I IFN signaling through the action of viral proteins (NS2A, NS4A, NS4B, and NS5) targeting cellular STAT signaling proteins [29, 30]. Various other viruses have also been shown to modify mitochondrial function either through direct interaction of viral proteins with mitochondrial components or indirectly via association with structures that interact with the mitochondria such as the mitochondria-associated membrane (MAM) fraction of the ER [31]. Whether DENV similarly directly modifies mitochondrial function has yet to be determined, but the DENV NS2B-3 protease has been shown to cleave the MAM-associated STING protein, thereby inhibiting the production of type I IFN [32]. DENV NS4A was shown to induce autophagy and enhance viral replication [23]. Inhibition of autophagy reduced DENV-2 infection, whereas maturation of autophagosomes to autolysosomes was important for DENV-3 replication [20, 22, 33]. These results suggest serotype-specific regulation of autophagosome maturation.

As noted above, studies have shown T cell activation in acute DENV infections, with higher levels of T cell activation markers such as CD69 and soluble CD4 and CD8 in patients with DHF [34]. The main targets of DENV infection, dendritic cells and monocytes, are important antigen-presenting cells. Autophagosomes can facilitate loading of antigen onto MHC class II molecules for presentation to CD4+ T cells [35-37] and can also cross-present antigen in the context of MHC class I molecules [38]. The regulation of autophagy by DENV may therefore influence the repertoire of epitopes presented by antigen-presenting cells and alter the type and intensity of the T cell response.



### **Kinetics and specificity of T lymphocyte responses during acute dengue virus infection**

The current understanding of immune responses to DENV infection has been driven by paradigms related to kinetics, frequency, and serotype-cross-reactivity. Due to the amino acid sequence homology among the four DENV serotypes, the kinetics of the adaptive immune response to secondary heterologous DENV infection have been presumed to reflect the preferential activation of cross-reactive memory T and B cells, i.e., more rapid and greater expansion and activation in secondary infection than in primary infection. In the absence of prior laboratory data, the pattern of serotype-cross-reactivity observed in secondary infection was therefore used to infer the serotype of previous DENV infections [39]. These assumptions have recently been challenged, however. Daily sampling and analysis of PBMC from patients with acute DENV infection revealed earlier peak frequencies of DENV-specific T cells in primary infection than in secondary infection [40]. Given the more rapid proliferation of memory T cells, and the more rapid clearance of viremia in secondary infection [8], this result was unexpected and requires revision of the model. As the kinetics of expansion and activation are critical to an effective immune response, this finding may reflect an effect of secondary heterologous DENV infection independent of viremia that delays T cell expansion, leading to an increased risk of severe disease. Seemingly contrary to a delay in T cell responses, however, is the finding that an earlier peak IFN- $\gamma$  response was seen in DHF versus DF cases [9]. This implies that the functional profile of DENV-reactive T cells is as important as their expansion to their influence on disease outcome, as has been suggested previously [41-43].

The relationship between the frequency of DENV-specific T lymphocytes and clinical outcome appears to be dependent on the epitope studied, among other factors. For example, T cell responses to HLA-A24-restricted [44] and HLA-B7-restricted [45] epitopes appeared to correlate with disease severity, whereas T cell responses to an HLA-B57-restricted epitope [46] did not. Perhaps the most studied DENV epitope, HLA-A11/NS3<sub>133</sub>, induced a tetramer-positive population that appeared to correlate with

disease outcome in one population of patients [47], but not others [40, 48], and cytokine responses, but not degranulation, to this epitope were associated with disease outcome [41]. Perhaps most intriguing was the finding that the frequency of HLA-B7-restricted tetramer-positive T cells appeared to correlate with disease severity only in HLA-A11-negative subjects and not in HLA-A11-positive individuals [40]. This supports an influential role for specific HLA haplotypes in crafting DENV-specific T cell responses that inform disease outcome [49].

Another recent finding challenging previous models was that the extent of DENV serotype-cross-reactivity among T cells was comparable in primary and secondary DENV infections [40]. While counterintuitive, this finding indicates that the T cell response is poised to respond to the variant epitopes encountered in secondary heterologous DENV infections. In the case of the HLA-A11-restricted CD8+ T cell epitope NS3<sub>133</sub> [39], some variants induced comparable responses while others induced rather poor responses [50]. This fits with the epidemiological data, in that many cases of DENV infection result in mild disease and others manifest with more severe disease, and supports the model that T cell responses can contribute to either protection or immunopathogenesis.

Some of the above findings have been replicated in mouse models of DENV infection. Sequential infection of immunocompetent mice with different DENV serotypes has recapitulated skewing of the DENV-specific T cell response [51, 52]. Additional studies have shown differences in the ability of epitope-specific T cells to clear antigen-presenting cells in vivo [53]. HLA-transgenic mice and immunodeficient mice reconstituted with human hematopoietic stem cells have been shown to recognize some of the same epitopes as humans with the corresponding HLA allotypes [54-56]. Given the challenges presented by heterogeneity of both HLA alleles and infection sequences in the setting of natural DENV infection in humans, these results raise the possibility that such mouse models can be used to further explore the immune response to secondary DENV infection, although the lack of a reliable mouse model of human dengue disease will necessitate cautious interpretation of the results.

## **Immunological memory in natural infection and vaccination- relation to outcomes**

In 2008, the World Health Organization Initiative for Vaccine Research convened an expert scientific panel to consult on cell-mediated immunity in dengue and DENV vaccine development [57]. A key recommendation of this committee was that there would be great interest in documenting the cellular response to DENV vaccination during clinical phase I-III vaccine testing to better understand the short- and long-term safety and immunogenicity of the vaccine candidates and potentially to define an immunological correlate of protection. Most focus has been placed on the role of neutralizing antibodies as a correlate of protection, without success (to date). A role for T cell responses in vaccine-mediated protection against flavivirus infection has been suggested by systems biology studies of recipients of the live attenuated yellow fever vaccine [58].

Evidence from a prospective dengue cohort study points to the potential for pre-existing DENV-specific memory T lymphocytes to either reduce or increase the severity of illness during a subsequent DENV infection. In this study, PBMC samples were obtained from a study cohort in an endemic region, and subjects were then monitored for the development of acute febrile illnesses [59, 60]. Serologic testing in the entire study cohort after each DENV transmission season also identified subjects who had serologic evidence of an intervening DENV infection despite having no recognized dengue illness (subclinical infections). Two immunology studies in this cohort have suggested that Th1-biased memory T cell responses dominated by IFN- $\gamma$  are associated with less severe secondary DENV infection. In the first study, IFN- $\gamma$  production by PBMC stimulated for 7 days with inactivated DENV antigens showed broader serotype-cross-reactivity in a subset of subjects with non-hospitalized symptomatic DENV infections than in subjects who were hospitalized by the treating clinician [61]. In the second study, the mean frequency of CD4+ T cells producing IFN- $\gamma$  in response to short-term (16-18 hrs) stimulation with inactivated DENV antigens was significantly higher in subjects who had subclinical DENV infection than in subjects who had a symptomatic DENV infection [62]. On the other hand, the same studies also

suggest that T cell production of  $\text{TNF}\alpha$ , which seems to be a preferential feature of serotype-cross-reactive responses [63], is associated with more severe infection. In the first study mentioned above,  $\text{TNF}\alpha$  was detected in culture supernatants of antigen-stimulated PBMC only from a subset of subjects who were hospitalized during the acute DENV infection [61]. This implies that this specific pattern of memory T cell responses represents a higher risk for more severe disease, but does not explain all severe dengue cases.

Several additional lines of evidence support the potential for memory DENV-specific T lymphocytes to contribute to protective immunity against severe dengue disease. While some HLA alleles are more frequently found in patients with severe dengue disease than in those with milder illness (or in the general population), other alleles are significantly less common in subjects with severe illness [49]. Furthermore, these positive and negative associations appear to be specific for secondary DENV infections and depend on the DENV serotype causing the secondary infection [64]. In a recent study of healthy adults in Sri Lanka, two-thirds of whom had serologic evidence of multiple prior DENV infections, the frequency of  $\text{IFN-}\gamma$ -secreting T cells measured in ELISPOT assays in response to DENV peptide stimulation showed a weak but statistically significant correlation with the ranking of that particular HLA allele in terms of susceptibility or resistance to severe disease [65]. This finding also supports a protective role for  $\text{IFN-}\gamma$  responses, as suggested by the cohort study described above.

Studies of T cell responses induced by DENV vaccines have mainly been confined to small phase I or phase II studies of live attenuated vaccines [66-70]. Similar to the findings in natural DENV infection, these vaccines induce predominantly Th1-type responses to multiple serotypes. In at least one study, these responses included polyfunctional T cells producing  $\text{IFN-}\gamma$ ,  $\text{TNF}\alpha$ , and IL-2, which have been associated with protective immunity for other infections [71-73]. For most of these studies, no association with protection could be evaluated because subjects were not known to have subsequent exposure to DENV. In one study, 10 subjects who had previously received a tetravalent live attenuated

vaccine were experimentally infected with either DENV-1 or DENV-3 [74]. All five subjects challenged with DENV-1 were protected, whereas only 2 of 5 subjects challenged with DENV-3 showed evidence of protective immunity. PBMC were collected from subjects at serial time points during the 14-day challenge period and tested for cytokine production in response to in vitro stimulation with DENV. PBMC from subjects who were protected from the challenge infection showed sustained production of IFN- $\gamma$ , whereas PBMC from subjects who were not protected lost this response by day 5 post-challenge.

### **Areas of uncertainty**

Current knowledge regarding the relationship between pre-existing DENV-specific immune responses and clinical outcome is based on a very limited number of subjects. Given the important role of host genetics in controlling immune responses, it will be important to substantially expand these analyses to additional populations in order to evaluate the generalizability of these findings and establish robust correlates of protective or pathological immunity. The immunological characterizations of acute dengue illness are skewed by the threshold of illness severity necessary for diagnosis; that is, the individuals who enrolled in those studies felt ill enough to seek medical attention. Comparatively little is known about the immune response in very mild (or asymptomatic) infections, although these might be most informative for an effective vaccine. Subjects with acute DENV infection demonstrate potent immune activation even at the earliest stages of clinical illness, meaning that the earliest immunological events, potentially of greatest impact on viral replication and disease, were missing from analysis. None of the studies published to date has evaluated the relationships between T cell responses present prior to secondary DENV infection and those detected during acute illness.

Additional uncertainties arise in relating any immunological correlates defined in the context of natural infection to those induced by vaccination. The DENV vaccine candidates currently in clinical trials reflect a variety of vaccine modalities, including recombinant proteins, virus-like particles (VLP), killed

inactivated viruses, DNA vaccines, live-attenuated dengue viruses, and live chimeric flaviviruses [75]. These vaccines differ in the DENV components included, the valency for each component (number of DENV serotypes included), and the formulation in which the components are presented to the immune system [34]. Protective mechanisms may not be the same for each of these vaccines. Killed inactivated or recombinant protein/VLP vaccines will likely generate a very different immune response from those generated by live-attenuated vaccines. Even subtle differences in the quality of the T cell response could potentially shift the balance of immunity from protection to disease enhancement. The observation of immune enhancement of disease with other virus vaccines provides a precedent for undesirable vaccine-induced immune responses [76]. Additionally, whereas natural infection almost always involves a single DENV serotype, all of the current vaccine candidates incorporate antigens from multiple serotypes. This aspect of vaccine design is likely to alter which T cell populations are preferentially activated and expanded. While potentially circumventing the phenomenon of “original antigenic sin,” at least in vaccinees who have not previously been exposed to DENV, there is insufficient evidence to predict the functional phenotypes of the responding T cell populations. Clinical studies seeking to define correlates of protective or pathological immunity will need to include subjects with a wide range of prior DENV exposure histories.

## **Conclusions**

The paradigm of severe dengue disease is that dengue-associated plasma leakage reflects an immune response to systemic DENV infection and is enhanced in patients with prior exposure to DENV. In this context, recent findings on innate and adaptive immune responses to DENV and their interactions have added several layers of complexity. Efforts to identify a single mechanism responsible for the plasma leakage phenomenon have not borne fruit. Instead, a number of immune responses have shown correlations with either less severe disease (protective immunity) or more severe disease (pathologic

immunity), and we are left with the possibility that multiple mechanisms might converge on the same clinical outcome. Further clinical studies of well-characterized patients with natural DENV infection continue to be highly relevant to resolving these areas of uncertainty. DENV vaccines currently in advanced clinical development differ sufficiently from natural DENV infection such that detailed immunologic characterization of vaccinees will be important to support the identification of immune markers or correlates of vaccine efficacy.

## **Figure legend**

Figure 1- Innate and adaptive immune responses to dengue virus and their relationships to protection or disease. Text boxes in the figure note immune responses activated in the infected cell (DENV-infected monocyte), in DENV-specific T lymphocytes that recognize DENV epitopes expressed by the infected cell, or in vascular endothelial cells. Green arrows denote those pathways that are primarily protective against infection or disease, red arrows denote those pathways that are primarily associated with more severe disease (pathological immune responses), and yellow arrows denote pathways that can be associated with either protection or pathogenesis, as described in the text. Monocytes (shown here), macrophages, and dendritic cells are the primary targets for dengue virus (DENV) infection; DENV infection of cells is enhanced by DENV-antibody complexes formed in the presence of pre-existing DENV-specific antibodies (not shown) from a previous DENV infection or passive transfer, e.g., in newborn infants. DENV-specific T lymphocytes responding to the current infection include both naïve T cells and serotype-cross-reactive memory T cells from a prior DENV infection. As illustrated in the figure, plasma leakage results from the action of circulating mediators on vascular endothelial cells, whereas direct infection of endothelial cells by DENV is not considered a major contributing factor in vivo.



## References

1. Sabin AB. Research on dengue during World War II. *Am J Trop Med Hyg.* 1952;1(1):30-50.
2. Halstead SB, Nimmannitya S, Cohen SN. Observations related to pathogenesis of dengue hemorrhagic fever. IV. Relation of disease severity to antibody response and virus recovered. *Yale J Biol Med.* 1970;42(5):311-28. PMID: 2591704.
3. Burke DS, Nisalak A, Johnson DE, Scott RM. A prospective study of dengue infections in Bangkok. *Am J Trop Med Hyg.* 1988;38(1):172-80.
4. Thein S, Aung MM, Shwe TN, Aye M, Zaw A, Aye K, Aye KM, Aaskov J. Risk factors in dengue shock syndrome. *Am J Trop Med Hyg.* 1997;56(5):566-72.
5. Endy TP, Yoon IK, Mammen MP. Prospective cohort studies of dengue viral transmission and severity of disease. *Curr Top Microbiol Immunol.* 2010;338:1-13.
6. Halstead SB. Observations related to pathogenesis of dengue hemorrhagic fever. VI. Hypotheses and discussion. *Yale J Biol Med.* 1970;42(5):350-62. PMID: 2591710.
7. Simmons CP, Farrar JJ, Nguyen v V, Wills B. Dengue. *N Engl J Med.* 2012;366(15):1423-32.
8. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Endy TP, Raengsakulrach B, Rothman AL, Ennis FA, Nisalak A. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis.* 2000;181(1):2-9.
9. Libraty DH, Endy TP, Houg HS, Green S, Kalayanarooj S, Suntayakorn S, Chansiriwongs W, Vaughn DW, Nisalak A, Ennis FA, Rothman AL. Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections. *J Infect Dis.* 2002;185(9):1213-21.
- \*10. Srikiatkachorn A, Wichit S, Gibbons RV, Green S, Libraty DH, Endy TP, Ennis FA, Kalayanarooj S, Rothman AL. Dengue viral RNA levels in peripheral blood mononuclear cells are associated with

disease severity and preexisting dengue immune status. PLoS ONE. 2012;7(12):e51335. PMCID: 3526575.

*This study examined the distribution of DENV RNA among PBMC subsets during acute infection. Cell-associated DENV RNA levels were highest in B lymphocytes and monocyte fractions and were significantly higher in secondary DENV infections.*

- \*11. Yoon IK, Rothman AL, Tannitisupawong D, Srikiatkachorn A, Jarman RG, Aldstadt J, Nisalak A, Mammen MP, Jr., Thammapalo S, Green S, Libraty DH, Gibbons RV, Getis A, Endy T, Jones JW, Koenraadt CJ, Morrison AC, Fansiri T, Pimgate C, Scott TW. Underrecognized mildly symptomatic viremic dengue virus infections in rural Thai schools and villages. J Infect Dis. 2012;206(3):389-98. PMCID: 3490697.

*This study enrolled individuals living near hospitalized dengue patients and detected subjects who were viremic but had minimal or no symptoms and maintained their normal daily activities. Such individuals in the community have the potential to contribute to DENV transmission.*

12. Srikiatkachorn A, Green S. Markers of dengue disease severity. Curr Top Microbiol Immunol. 2010;338:67-82.

13. Popper SJ, Gordon A, Liu M, Balmaseda A, Harris E, Relman DA. Temporal dynamics of the transcriptional response to dengue virus infection in Nicaraguan children. PLoS neglected tropical diseases. 2012;6(12):e1966. PMCID: 3527342.

14. Simmons CP, Popper S, Dolocek C, Chau TN, Griffiths M, Dung NT, Long TH, Hoang DM, Chau NV, Thao le TT, Hien TT, Relman DA, Farrar J. Patterns of host genome--wide gene transcript abundance in the peripheral blood of patients with acute dengue hemorrhagic fever. J Infect Dis. 2007;195(8):1097-107.

15. Green S, Pichyangkul S, Vaughn DW, Kalayanarooj S, Nimmannitya S, Nisalak A, Kurane I, Rothman AL, Ennis FA. Early CD69 expression on peripheral blood lymphocytes from children with dengue hemorrhagic fever. *J Infect Dis.* 1999;180:1429-35.
16. Libraty DH, Endy TP, Hough HH, Green S, Kalayanarooj S, Suntayakorn S, Vaughn DW, Nisalak A, Rothman AL, Ennis FA. Differing influences of viral burden and immune activation on disease severity in secondary dengue 3 virus infections. *J Infect Dis.* 2002;185:1213-21.
17. Welsch S, Miller S, Romero-Brey I, Merz A, Bleck CK, Walther P, Fuller SD, Antony C, Krijnse-Locker J, Bartenschlager R. Composition and three-dimensional architecture of the dengue virus replication and assembly sites. *Cell Host Microbe.* 2009;5(4):365-75.
18. Miller S, Krijnse-Locker J. Modification of intracellular membrane structures for virus replication. *Nature reviews Microbiology.* 2008;6(5):363-74.
19. Heaton NS, Perera R, Berger KL, Khadka S, Lacount DJ, Kuhn RJ, Randall G. Dengue virus nonstructural protein 3 redistributes fatty acid synthase to sites of viral replication and increases cellular fatty acid synthesis. *Proc Natl Acad Sci U S A.* 2010;107(40):17345-50. PMID: 2951450.
20. Lee YR, Lei HY, Liu MT, Wang JR, Chen SH, Jiang-Shieh YF, Lin YS, Yeh TM, Liu CC, Liu HS. Autophagic machinery activated by dengue virus enhances virus replication. *Virology.* 2008;374(2):240-8.
21. Panyasrivanit M, Khakpoor A, Wikan N, Smith DR. Co-localization of constituents of the dengue virus translation and replication machinery with amphisomes. *J Gen Virol.* 2009;90(Pt 2):448-56.
22. Khakpoor A, Panyasrivanit M, Wikan N, Smith DR. A role for autophagolysosomes in dengue virus 3 production in HepG2 cells. *J Gen Virol.* 2009;90(Pt 5):1093-103.
- \*23. McLean JE, Wudzinska A, Datan E, Quaglino D, Zakeri Z. Flavivirus NS4A-induced autophagy protects cells against death and enhances virus replication. *The Journal of biological chemistry.* 2011;286(25):22147-59. PMID: 3121359.

*This report showed that the DENV NS4A protein induced PI3K-dependent autophagy and protected cells against death during infection.*

24. Chang TH, Liao CL, Lin YL. Flavivirus induces interferon-beta gene expression through a pathway involving RIG-I-dependent IRF-3 and PI3K-dependent NF-kappaB activation. *Microbes Infect.* 2006;8(1):157-71.
25. Loo YM, Fornek J, Crochet N, Bajwa G, Perwitasari O, Martinez-Sobrido L, Akira S, Gill MA, Garcia-Sastre A, Katze MG, Gale M, Jr. Distinct RIG-I and MDA5 signaling by RNA viruses in innate immunity. *J Virol.* 2008;82(1):335-45. PMID: 2224404.
26. Zhong B, Yang Y, Li S, Wang YY, Li Y, Diao F, Lei C, He X, Zhang L, Tien P, Shu HB. The adaptor protein MITA links virus-sensing receptors to IRF3 transcription factor activation. *Immunity.* 2008;29(4):538-50.
27. Lee HK, Lund JM, Ramanathan B, Mizushima N, Iwasaki A. Autophagy-dependent viral recognition by plasmacytoid dendritic cells. *Science.* 2007;315(5817):1398-401.
28. Mackenzie J. Wrapping things up about virus RNA replication. *Traffic.* 2005;6(11):967-77.
29. Kou Z, Quinn M, Chen H, Rodrigo WW, Rose RC, Schlesinger JJ, Jin X. Monocytes, but not T or B cells, are the principal target cells for dengue virus (DV) infection among human peripheral blood mononuclear cells. *J Med Virol.* 2008;80(1):134-46.
30. Munoz-Jordan JL, Sanchez-Burgos GG, Laurent-Rolle M, Garcia-Sastre A. Inhibition of interferon signaling by dengue virus. *Proc Natl Acad Sci U S A.* 2003;100(24):14333-8.
31. D'Agostino DM, Bernardi P, Chieco-Bianchi L, Ciminale V. Mitochondria as functional targets of proteins coded by human tumor viruses. *Advances in cancer research.* 2005;94:87-142.
- \*\*32. Aguirre S, Maestre AM, Pagni S, Patel JR, Savage T, Gutman D, Maringer K, Bernal-Rubio D, Shabman RS, Simon V, Rodriguez-Madoz JR, Mulder LC, Barber GN, Fernandez-Sesma A. DENV

inhibits type I IFN production in infected cells by cleaving human STING. *PLoS Pathog.* 2012;8(10):e1002934. PMID: 3464218.

*This paper identified human STING as a target of the DENV NS2B3 protease complex and showed that cleavage of STING by NS2B3 was involved in inhibition of IFN induction.*

33. Panyasrivanit M, Khakpoor A, Wikan N, Smith DR. Co-localization of constituents of the dengue virus translation and replication machinery with amphisomes. *J Gen Virol.* 2009;90(Pt 2):448-56.
34. Rothman AL. Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. *Nat Rev Immunol.* 2011;11(8):532-43.
35. Nimmerjahn F, Milosevic S, Behrends U, Jaffee EM, Pardoll DM, Bornkamm GW, Mautner J. Major histocompatibility complex class II-restricted presentation of a cytosolic antigen by autophagy. *European journal of immunology.* 2003;33(5):1250-9.
36. Dengjel J, Schoor O, Fischer R, Reich M, Kraus M, Muller M, Kreymborg K, Altenberend F, Brandenburg J, Kalbacher H, Brock R, Driessen C, Rammensee HG, Stevanovic S. Autophagy promotes MHC class II presentation of peptides from intracellular source proteins. *Proceedings of the National Academy of Sciences of the United States of America.* 2005;102(22):7922-7. PMID: 1142372.
37. Schmid D, Pypaert M, Munz C. Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. *Immunity.* 2007;26(1):79-92. PMID: 1805710.
38. Li Y, Wang LX, Yang G, Hao F, Urba WJ, Hu HM. Efficient cross-presentation depends on autophagy in tumor cells. *Cancer research.* 2008;68(17):6889-95. PMID: 2905686.
39. Mongkolsapaya J, Dejnirattisai W, Xu XN, Vasanawathana S, Tangthawornchaikul N, Chairunsri A, Sawasdivorn S, Duangchinda T, Dong T, Rowland-Jones S, Yenichitsomanus PT, McMichael A,

- Malasit P, Screaton G. Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. *Nat Med.* 2003;9(7):921-7.
- \*\*40. Friberg H, Bashyam H, Toyosaki-Maeda T, Potts JA, Greenough T, Kalayanarooj S, Gibbons RV, Nisalak A, Srikiatkachorn A, Green S, Stephens HA, Rothman AL, Mathew A. Cross-reactivity and expansion of dengue-specific T cells during acute primary and secondary infections in humans. *Sci Rep.* 2011;1:51. PMID: 3216538.
- The study involved a comprehensive analysis of tetramer-specific T cells in blood samples collected during acute primary and secondary DENV infections. Data from this study challenge previous models of T cell expansion kinetics, activation, and serotype-cross-reactivity.*
- \*41. Duangchinda T, Dejnirattisai W, Vasanawathana S, Limpitikul W, Tangthawornchaikul N, Malasit P, Mongkolsapaya J, Screaton G. Immunodominant T-cell responses to dengue virus NS3 are associated with DHF. *Proc Natl Acad Sci U S A.* 2010;107(39):16922-7.
- This study involved the assessment of DENV-specific T cell responses in primary and secondary infection with respect to disease outcome. The data demonstrate that T cell functional profiles differ in mild versus severe disease.*
42. Dong T, Moran E, Vinh Chau N, Simmons C, Luhn K, Peng Y, Wills B, Phuong Dung N, Thi Thu Thao L, Hien TT, McMichael A, Farrar J, Rowland-Jones S. High pro-inflammatory cytokine secretion and loss of high avidity cross-reactive cytotoxic T-cells during the course of secondary dengue virus infection. *PLoS ONE.* 2007;2(12):e1192.
43. Mangada MM, Endy TP, Nisalak A, Chunsuttiwat S, Vaughn DW, Libraty DH, Green S, Ennis FA, Rothman AL. Dengue-specific T cell responses in peripheral blood mononuclear cells obtained prior to secondary dengue virus infections in Thai schoolchildren. *J Infect Dis.* 2002;185(12):1697-703.

44. Mongkolsapaya J, Duangchinda T, Dejnirattisai W, Vasanawathana S, Avirutnan P, Jairungsri A, Khemnu N, Tangthawornchaikul N, Chotiyarnwong P, Sae-Jang K, Koch M, Jones Y, McMichael A, Xu X, Malasit P, Screaton G. T cell responses in dengue hemorrhagic fever: are cross-reactive T cells suboptimal? *J Immunol.* 2006;176(6):3821-9.
45. Zivna I, Green S, Vaughn DW, Kalayanarooj S, Stephens HAF, Chandanayingyong D, Nisalak A, Ennis FA, Rothman AL. T cell responses to an HLA B\*07-restricted epitope on the dengue NS3 protein correlate with disease severity. *J Immunol.* 2002;168:5959-65.
- \*46. Townsley E, Woda M, Thomas SJ, Kalayanarooj S, Gibbons RV, Nisalak A, Srikiatkachorn A, Green S, Stephens HA, Rothman AL, Mathew A. Distinct Activation Phenotype of a Highly Conserved Novel HLA-B57-Restricted Epitope during Dengue Virus Infection. *Immunology.* 2013. *This study identified a highly conserved epitope on the DENV NS1 protein. Flow cytometry analyses demonstrated that CD71 was a novel marker of T cell activation specific for tetramer-positive T cells.*
47. Mongkolsapaya J, Dejnirattisai W, Xu X, Vasanawathana S, Tangthawornchaikul N, Chairunsri A, Sawasdivorn S, Duangchinda T, Dong T, Rowland-Jones S, Yenichitsomanus P, McMichael A, Malasit P, Screaton G. Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. *Nature Med.* 2003;9(7):921-7.
48. Dung NT, Duyen HT, Thuy NT, Ngoc TV, Chau NV, Hien TT, Rowland-Jones SL, Dong T, Farrar J, Wills B, Simmons CP. Timing of CD8+ T cell responses in relation to commencement of capillary leakage in children with dengue. *J Immunol.* 2010;184(12):7281-7.
49. Stephens HA. HLA and other gene associations with dengue disease severity. *Curr Top Microbiol Immunol.* 2010;338:99-114.

50. Friberg H, Burns L, Woda M, Kalayanarooj S, Endy TP, Stephens HA, Green S, Rothman AL, Mathew A. Memory CD8+ T cells from naturally acquired primary dengue virus infection are highly cross-reactive. *Immunol Cell Biol.* 2011;89(1):122-9. PMID: 2929403.
51. Beaumier CM, Mathew A, Bashyam HS, Rothman AL. Cross-reactive memory CD8(+) T cells alter the immune response to heterologous secondary dengue virus infections in mice in a sequence-specific manner. *J Infect Dis.* 2008;197(4):608-17.
52. Beaumier CM, Rothman AL. Cross-reactive memory CD4+ T cells alter the CD8+ T-cell response to heterologous secondary dengue virus infections in mice in a sequence-specific manner. *Viral Immunol.* 2009;22(3):215-9.
53. Beaumier CM, Jaiswal S, West KY, Friberg H, Mathew A, Rothman AL. Differential in vivo clearance and response to secondary heterologous infections by H2(b)-restricted dengue virus-specific CD8+ T cells. *Viral Immunol.* 2010;23(5):477-85.
54. Weiskopf D, Yauch LE, Angelo MA, John DV, Greenbaum JA, Sidney J, Kolla RV, De Silva AD, de Silva AM, Grey H, Peters B, Shrestha S, Sette A. Insights into HLA-restricted T cell responses in a novel mouse model of dengue virus infection point toward new implications for vaccine design. *J Immunol.* 2011;187(8):4268-79. PMID: 3186824.
55. Jaiswal S, Pearson T, Friberg H, Shultz LD, Greiner DL, Rothman AL, Mathew A. Dengue virus infection and virus-specific HLA-A2 restricted immune responses in humanized NOD-scid IL2rgammanull mice. *PLoS ONE.* 2009;4(10):e7251.
56. Jaiswal S, Pazoles P, Woda M, Shultz LD, Greiner DL, Brehm MA, Mathew A. Enhanced humoral and HLA-A2-restricted dengue virus-specific T-cell responses in humanized BLT NSG mice. *Immunology.* 2012;136(3):334-43. PMID: 3385033.
57. Thomas SJ, Hombach J, Barrett A. Scientific consultation on cell mediated immunity (CMI) in dengue and dengue vaccine development. *Vaccine.* 2009;27(3):355-68.



58. Querec TD, Akondy RS, Lee EK, Cao W, Nakaya HI, Teuwen D, Pirani A, Gernert K, Deng J, Marzolf B, Kennedy K, Wu H, Bennouna S, Oluoch H, Miller J, Vencio RZ, Mulligan M, Aderem A, Ahmed R, Pulendran B. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. *Nature immunology*. 2009;10(1):116-25.
59. Endy TP, Chunsuttiwat S, Nisalak A, Libraty DH, Green S, Rothman AL, Vaughn DW, Ennis FA. Epidemiology of inapparent and symptomatic acute dengue virus infection: a prospective study of primary school children in kampaeng phet, Thailand. *AmJEpidemiol*. 2002;156(1):40-51.
60. Yoon IK, Rothman AL, Tannitisupawong D, Srikiatkachorn A, Jarman RG, Aldstadt J, Nisalak A, Mammen MP, Jr., Thammapalo S, Green S, Libraty DH, Gibbons RV, Getis A, Endy T, Jones JW, Koenraadt CJ, Morrison AC, Fansiri T, Pimgate C, Scott TW. Underrecognized mildly symptomatic viremic dengue virus infections in rural Thai schools and villages. *The Journal of infectious diseases*. 2012;206(3):389-98. PMID: 3490697.
61. Mangada MM, Endy TP, Nisalak A, Chunsuttiwat S, Vaughn DW, Libraty DH, Green S, Ennis FA, Rothman AL. Dengue-specific T cell responses in peripheral blood mononuclear cells obtained prior to secondary dengue virus infections in Thai schoolchildren. *JInfectDis*. 2002;185(12):1697-703.
- \*\*62. Hatch S, Endy TP, Thomas S, Mathew A, Potts J, Pazoles P, Libraty DH, Gibbons R, Rothman AL. Intracellular cytokine production by dengue virus-specific T cells correlates with subclinical secondary infection. *J Infect Dis*. 2011;203:1282-91.
- This study measured the frequencies of DENV-specific memory T cells in PBMC collected prior to secondary DENV infections. The data showed significantly higher IFN  $\gamma$ -producing T cell frequencies in subjects who had subclinical secondary infections.*
63. Mangada MM, Rothman AL. Altered cytokine responses of dengue-specific CD4+ T cells to heterologous serotypes. *J Immunol*. 2005;175(4):2676-83.

64. Stephens HA, Klaythong R, Sirikong M, Vaughn DW, Green S, Kalayanarooj S, Endy TP, Libraty DH, Nisalak A, Innis BL, Rothman AL, Ennis FA, Chandanayingyong D. HLA-A and -B allele associations with secondary dengue virus infections correlate with disease severity and the infecting viral serotype in ethnic Thais. *Tissue Antigens*. 2002;60(4):309-18.
- \*65. Weiskopf D, Angelo MA, de Azeredo EL, Sidney J, Greenbaum JA, Fernando AN, Broadwater A, Kolla RV, De Silva AD, de Silva AM, Mattia KA, Doranz BJ, Grey HM, Shrestha S, Peters B, Sette A. Comprehensive analysis of dengue virus-specific responses supports an HLA-linked protective role for CD8+ T cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;110(22):E2046-53. PMID: 3670335.
- This study tested for IFN $\gamma$  ELISPOT responses to an extensive panel of DENV peptides in PBMC from healthy adults from Sri Lanka. The data show significantly higher frequencies of T cells that are restricted by HLA alleles that have shown associations with resistance to severe dengue illness.*
66. Rothman AL, Kanesa-thasan N, West K, Janus J, Saluzzo J, Ennis FA. Induction of T lymphocyte responses to dengue virus by a candidate tetravalent live attenuated dengue virus vaccine. *Vaccine*. 2001;19(32):4694-9.
67. Gwinn W, Sun W, Innis BL, Caudill J, King AD. Serotype-specific T(H)1 responses in recipients of two doses of candidate live-attenuated dengue virus vaccines. *Am J Trop Med Hyg*. 2003;69(6 Suppl):39-47.
68. Guy B, Nougarede N, Begue S, Sanchez V, Souag N, Carre M, Chambonneau L, Morrisson DN, Shaw D, Qiao M, Dumas R, Lang J, Forrat R. Cell-mediated immunity induced by chimeric tetravalent dengue vaccine in naive or flavivirus-primed subjects. *Vaccine*. 2008;26(45):5712-21.

69. Lindow JC, Durbin AP, Whitehead SS, Pierce KK, Carmolli MP, Kirkpatrick BD. Vaccination of volunteers with low-dose, live-attenuated, dengue viruses leads to serotype-specific immunologic and virologic profiles. *Vaccine*. 2013;31(33):3347-52. PMID: 3777849.
70. Harenberg A, Begue S, Mamessier A, Gimenez-Fourage S, Ching Seah C, Wei Liang A, Li Ng J, Yun Toh X, Archuleta S, Wilder-Smith A, Shek LP, Wartel-Tram A, Bouckennooghe A, Lang J, Crevat D, Caillet C, Guy B. Persistence of Th1/Tc1 responses one year after tetravalent dengue vaccination in adults and adolescents in Singapore. *Hum Vaccin Immunother*. 2013;9(11).
71. Thakur A, Pedersen LE, Jungersen G. Immune markers and correlates of protection for vaccine induced immune responses. *Vaccine*. 2012;30(33):4907-20.
72. Tan AC, Eriksson EM, Kedzierska K, Deliyannis G, Valkenburg SA, Zeng W, Jackson DC. Polyfunctional CD8(+) T cells are associated with the vaccination-induced control of a novel recombinant influenza virus expressing an HCV epitope. *Antiviral Res*. 2012;94(2):168-78.
73. Betts MR, Harari A. Phenotype and function of protective T cell immune responses in HIV. *Curr Opin HIV AIDS*. 2008;3(3):349-55.
- \*\*74. Gunther VJ, Putnak R, Eckels KH, Mammen MP, Scherer JM, Lyons A, Szein MB, Sun W. A human challenge model for dengue infection reveals a possible protective role for sustained interferon gamma levels during the acute phase of illness. *Vaccine*. 2011;29(22):3895-904.  
*This study measured DENV-specific cytokine responses in 10 subjects who had received a tetravalent DENV vaccine and were then experimentally challenged with DENV-1 or DENV-3. Subjects who had protective immunity against the experimental challenge were characterized by the sustained ability to produce IFN $\gamma$  during the 14-day period after challenge.*
75. Thomas SJ, Endy TP. Vaccines for the prevention of dengue: development update. *Human vaccines*. 2011;7(6):674-84.

76. Polack FP. Atypical measles and enhanced respiratory syncytial virus disease (ERD) made simple.  
Pediatric research. 2007;62(1):111-5.