8-15-2020

T lymphocyte responses to flaviviruses — diverse cell populations affect tendency toward protection and disease

Luis Alberto Sanchez Vargas

Anuja Mathew
University of Rhode Island, mathewa@uri.edu

Alan L. Rothman
University of Rhode Island, alan_rothman@uri.edu

Citation/Publisher Attribution

Sanchez Vargas LA, Mathew A, Rothman AL. T lymphocyte responses to flaviviruses — diverse cell populations affect tendency toward protection and disease. Current Opinion in Virology 2020;43:28-34.

T lymphocyte responses to flaviviruses - diverse cell populations affect tendency toward protection and disease

Luis Alberto Sanchez Vargas, Anuja Mathew, Alan L Rothman

Institute for Immunology and Informatics and Department of Cell and Molecular Biology, University of Rhode Island, 80 Washington Street, Providence, RI 02903 USA

Corresponding author: Dr. Alan L Rothman, University of Rhode Island, 80 Washington St., Providence, RI 02903 USA, tel- 401-277-5408, email- alan_rothman@uri.edu

Declarations of interest: Drs. Sanchez, Mathew, and Rothman report grants from NIH/NIAID during the conduct of this work.

Abstract

Dengue virus (DENV), Yellow Fever virus, West Nile virus, Japanese encephalitis virus and Zika virus are medically important flaviviruses transmitted to humans by mosquitoes and circulate in overlapping geographic areas. Cross-reactive immune responses have been demonstrated among the flaviviruses, particularly the four DENV serotypes. The immunological imprint left by a flavivirus infection can therefore have profound effects on the responses to subsequent infections. In this review we summarize recent research focusing on T cell responses to DENV using clinical samples from prospective cohort studies in Asia. These data suggest that durability of different T cell populations after natural infection or vaccination is an important consideration for the outcome of subsequent flavivirus exposures and we argue for continued investigation in the context of longitudinal cohort studies.

Introduction

The flavivirus genus in the *Flaviviridae* family of positive-strand RNA viruses includes over 33 viruses known to infect humans [1]. Among these, the four dengue virus (DENV) serotypes, DENV1-4, represent a major global health problem, infecting an estimated 400 million individuals each year [2]. A world-wide pandemic of Zika virus (ZIKV) infections occurred from 2015-2016, and there remains the potential for future large outbreaks. Since these five viruses are transmitted by the same mosquito vectors, *Aedes aegypti* and *Aedes albopictus*, the population living in affected areas remain at risk for repeated exposures over their lifetime. Additionally, the geographic distribution of DENV overlaps substantially with that of other flaviviruses, such as yellow fever virus (YFV, which shares the same mosquito vector), West Nile virus (WNV), and Japanese encephalitis virus (JEV).

The flaviviruses vary in the degree of genetic relatedness, with the four DENV serotypes forming a distinct cluster in the phylogenetic tree. ZIKV is next most closely related to the DENV group, with YFV, WNV, and JEV having greater genetic divergence. The genetic relatedness among these viruses, discovered through the application of high-throughput sequencing, explains the serologic cross-reactivity among these viruses that was reported over 50 years earlier by Casals and others [3]. Serologic studies also established the concept that one flavivirus infection significantly modified the immune response to any subsequent flavivirus infection, resulting in high-titer cross-reactive antibody responses. These observations then led to the insight that severe dengue illness was strongly associated with secondary DENV infections [4].

We and other cellular immunologists recognized the potential parallels between these serologic observations and T lymphocyte responses to flaviviruses, and, further, the diversity of functional responses possible as a result of activation of cross-reactive memory T cells during a second

flavivirus infection [5–7]. Initial characterizations of flavivirus-reactive T cells confirmed antigenic cross-reactivity as well as altered responses after secondary flavivirus infection. As has been the case for antibodies, these studies provided only circumstantial evidence of a potential important contribution of cellular immunity to flavivirus infection outcomes. Our recent studies have sought to establish the clinical relevance of these responses to better understand individual risk factors for severe dengue illness and correlates of vaccine efficacy and safety.

Technical considerations in studies of flavivirus-specific T cell immunity

A major focus of our attention in recent years has been on study design and its consequences for the interpretation of results. Experimental flavivirus infection models have offered the greatest level of control over important parameters, e.g., the timing of viral challenge and sampling, size of challenge inoculum, and genetic background of the host. These models have enabled studies demonstrating, e.g., the impact of specific infection sequences and interactions with host genetics on both acute and memory phases of the T cell response [8]. However, the failure of most laboratory mice to faithfully recapitulate human infection and disease has been problematic for translation of these findings to humans. While a number of innovative models have been developed to address this limitation, such as “humanized” mouse strains and experimental infection of humans with modified viruses, studies of natural flavivirus infection remain an essential element of the research agenda [9].

Table 1 summarizes several key considerations in the design of observational studies of cellular immunity to flavivirus infections, and their implications for interpretation of study results. Several factors address the selection of subjects and specimens for analysis. Many studies have used blood samples collected either during or after an acute flavivirus infection, due to the ready availability of suitable patient populations in flavivirus-endemic areas [6,10–14]. This study design simplifies

the collection of specimens for detailed immunological characterization, and, with high-quality associated clinical data, identifies associations with illness and generates hypotheses regarding causality. However, it is clear that key immunological events precede even studies of the early phase of illness; a prospective cohort study design is therefore essential to explore the role of immune responses as predictors of risk. Clinical data available for statistical analyses is dependent on the sample collection strategy, and also introduces additional complexity. A common comparison, subjects with mild versus severe illness, relies on clear and consistently-applied clinical definitions. Mistakes in applying the criteria for dengue fever (DF) and dengue hemorrhagic fever (DHF) have been cited as an issue, but the newer classifications of dengue with warning signs (DWS) and severe dengue (SD) have in many ways been more problematic, as illustrated by the finding that antibody titers were more strongly associated with the earlier classification [15]. We have preferred the DF/DHF classification for symptomatic infections, which more clearly emphasizes plasma leakage as the principal component of more severe illness. We have also used continuous measures of plasma leakage, thrombocytopenia, and liver injury; this has allowed us to identify immunological associations with particular disease indicators [16].

The functional responses measured and their cross-reactivity for different flaviviruses are determined by the assay format and selection of antigens. A thorough treatment of these technical issues is beyond the scope of the current review, but several examples of relevant considerations are listed in Table 1. In particular, our strategy has been to measure the responses to individual viruses, e.g., DENV1-4, rather than to develop a single measure, as in the megapool strategy, because the distribution of different clinical outcomes is critically dependent on the specific virus with which each individual is infected.

Table 1. Considerations in the design of observational studies of cellular immunity in flavivirus infections.

Factor	Options	Implications
Timing of sample collection	Pre-infection During acute infection Post-infection	Pre-infection responses have predictive potential; acute responses reflect pathophysiology +/- pathogenesis
Clinical phenotypes	Severe illness Mild illness Inapparent infection Exposed without infection	Severe versus mild illness reflects pathophysiology, but both involve productive infection; inapparent dependent on surveillance, difficult to define exposure
T cell functions	Activation Cytokine production- IFNγ, TNFα, others Cytolysis	Effector functions that are most protective not well defined; many T cell functions also have potential role in disease pathogenesis
Antigen stimulation	No stimulation v stimulation Peptides v virus/infected cell Whole proteome v selected Single v multiple viruses	Measures the presence or function of T cells; affects percentage of virus-specific T cell repertoire detected (based on antigen specificity and/or T cell subset)

Cross-reactivity and functional diversity of flavivirus-reactive T cells

T cells are highly heterogeneous in peptide sequence specificity and functional responses. These are particularly important considerations for flavivirus-specific T cells given the potential implications for secondary flavivirus infections. Recipients of monovalent live flavivirus vaccines have been useful to characterize these responses due to their ability to donate large volumes of blood and well-defined virus exposure, often without confounding exposures to other flaviviruses [17,18]. Multiparameter flow cytometry of antigen-stimulated memory T cells has shown heterogeneous cytokine production at the single cell level for both CD4 [19] and CD8 [20] T cells. Although overall the highest frequencies of cytokine-producing T cells were observed in response to the homologous DENV serotype, in the case of some epitopes higher responses were observed to heterologous peptides. Additionally, the relative frequencies of T cells producing IFNγ versus TNFα or MIP-1β varied for different epitope variants. These data have demonstrated the potential for flavivirus antigens to act as altered peptide ligands for memory T cells during infection in individuals who have had a prior heterologous flavivirus exposure.

Although vaccines offer advantages as model live flavivirus infections, associations with infrequent clinical phenotypes and with sequential infection have continued to require the study of natural infections with wild-type viruses. In addition to a careful and thorough characterization of study subjects, we have found it important to analyze sequential blood samples starting before the critical phase of illness. Using flow cytometry, we have demonstrated that multiple populations of CD4 and CD8 T cells are activated and expanded during acute infection, and that this expansion precedes the development of plasma leakage [6,12]. In one recent study [12], we found a higher number of activated pT_{FH} cells during the critical phase of illness in subjects with secondary infections and those with DHF; the frequency of activated pT_{FH} cells also correlated with antibody

responses. While most studies from our group and others have pointed to a positive association between immune activation and disease severity, the direction of causality has remained a point of controversy.

Associations of pre-existing flavivirus-reactive T cell responses with clinical phenotype

Our efforts to address this causal relationship has directed our efforts towards the analysis of blood samples collected through prospective cohort studies. These studies have encountered several challenges, such as the low frequency of severe clinical phenotypes, limited blood samples from young subjects, and the large number of immune responses of interest based on the studies of acute infection. We have therefore applied several different laboratory methods (Table 2).

In our first study [19], we selected subjects with symptomatic secondary DENV infections. PBMC were stimulated with inactivated DENV antigens and we compared proliferation and cytokine secretion in subjects with hospitalized (more severe) versus non-hospitalized (less severe) dengue. There was no significant difference in T cell proliferation, however, IFN-γ production to multiple serotypes was associated with milder illness and TNFα production was associated with more severe illness. In our second study [21], we used the same stimulation method but measured the frequency of cytokine-producing CD4 T cells by flow cytometry. We compared responses in subjects with subclinical infection to those in subjects with symptomatic infection (regardless of severity of illness). This study found that subjects with subclinical infection had higher frequencies of CD4 T cells producing IFN-γ or IL-2 in response to DENV antigen than subjects with symptomatic infection. To extend these findings, we selected another subset of subjects with subclinical and symptomatic infections, and measured both the frequencies of responding cells as well as cytokine secretion in response to stimulation in vitro [22]. This study confirmed the association of higher frequencies of IFN-γ-producing CD4 T cells with subclinical infection. We

found that the secretion of IL-6, IL-15 and MCP-1 was higher by PBMC from children who developed symptomatic DENV infection, whereas the secretion of IL-12, IL-2R, MIP-1-α, RANTES, GM-CSF, and TNF-α was lower by PBMC from these children. These studies paint a complex picture of opposing positive/protective and negative/pathological effects of pre-existing flavivirus-specific T cell responses on the outcome of secondary DENV infection.

Table 2. Comparison of studies to identify clinical associations of dengue virus-specific immune responses measured in blood samples collected prior to secondary flavivirus infections.

Study	Clinical phenotypes	In vitro stimulation	Measurements
Mangada et al [19]	Hospitalized versus non-hospitalized dengue	Inactivated DENV-infected cell lysate (7 d)	Proliferation IFN-γ, TNFα secretion
Hatch et al [21]	Subclinical versus symptomatic DENV infection	Inactivated DENV-infected cell lysate (24 h)	% cytokine-positive CD4 T cells (IFN-γ, TNFα, IL-2)
Friberg et al [22]	Subclinical versus symptomatic DENV infection	Inactivated DENV-infected cell lysate (24 h)	% IFN-γ-positive CD4 T cells
		Infectious DENV (7 d)	Cytokine secretion

Temporal changes in responses of flavivirus-reactive T cells

Most studies of flavivirus-specific memory T cells, including those described above, have based their conclusions on a single time point. However, the stability of T cell memory has not been well characterized, particularly in settings where frequent re-exposures are possible. We have recently leveraged the longitudinal nature of our cohort study to address this gap. We measured DENV-specific T cell responses by IFNγ/IL-2 ELISPOT assays in PBMC collected over 5 consecutive years from individuals who had a symptomatic, subclinical or no DENV infection [23]; subclinical and no infection groups were defined serologically. DENV-specific T cells were low or absent before symptomatic secondary infection. Increased responses were observed following the infection, however, they fluctuated over the 3-4 years after infection. We observed some increases in T cell responses in the absence of serologic evidence of infection, raising the possibility of inapparent viral exposures. Furthermore, some subjects, particularly those who did not experience a symptomatic DENV infection during the study, showed greater stability in immune responses.

The above findings have led us to hypothesize that the durability of flavivirus-specific T cell responses after an infection and the cytokine responses of T cells that persist at low frequency may represent critical determinants of the outcome of a subsequent infection. Recently we have been studying the kinetics of T cell responses over multiple years prior to infection in subjects with mild (non-hospitalized) or severe (DHF) illness from our prospective cohort study [23]. To increase the sensitivity for low-frequency responses, we are using cultured ELISPOT assays [24]. Figure 1 shows two examples of responses over time to a structural protein peptide pool (less serotype-crossreactive) and a non-structural protein peptide pool (more serotype-crossreactive) in individuals who had a symptomatic DENV-2 infection in 2001. IFNγ T cell responses were low in 2001; in one subject, this followed a decline over several years in responses to non-structural

proteins. Post-infection, responses were significantly increased to both structural and non-structural proteins.

Implications for flavivirus vaccines

Applying the lessons from natural flavivirus infections to improve the design of vaccine products and dosing regimens remains a key goal for us and many others. There are currently a significant number of flavivirus vaccines in clinical testing [25,26]. We and others have characterized the flavivirus-specific T cell responses induced by different formulations and regimens of these vaccines [27,28]. Friberg et al demonstrated the complementary information that can be obtained from applying a suite of immune responses assays, including ELISPOT, cytokine flow cytometry, proliferation, and cytokine secretion assays [29]. Detailed single-cell analyses have demonstrated the power to identify distinct gene expression and metabolic features of T cell destined to persist into the memory phase [30]. However, the cohort studies described above suggest a cautious approach to translating those findings to children in flavivirus-endemic areas, where blood samples volumes are more limited and both pre-existing immunity and frequent re-exposures are likely. We are applying the lessons we have learned from cohort studies of natural infection to a cohort of Filipino children who received Dengvaxia^{TM} as part of the phase III efficacy trial. Figure 1 shows examples of apparent vaccine responses. Pre-vaccination PBMC in these cases showed low flavivirus-specific T cell responses, as was observed in our Thai cohort study; post-vaccination PBMC showed responses to some DENV structural proteins and YFV NS3 protein, but not to DENV non-structural proteins. Follow-up of this cohort will determine whether vaccine-induced immune responses are associated with protection against subsequent DENV infection.

Remaining questions

Recent studies have greatly advanced the characterization of flavivirus-specific T cell responses during both acute and memory phases. As illustrated in Figure 2, many functionally distinct T cell subpopulations are now known to be recruited into the acute response to natural infection and persist into memory, where they are capable of influencing the outcome of subsequent infections. Host genetic factors influence both the magnitude and specificity of this response. Qualitatively and quantitatively similar responses have also been observed in the acute and early memory phases after immunization of some, but not all vaccine candidates.

At the same time that technological advances have enhanced the detail in our understanding of components of T cell responses to flavivirus infection and vaccination, we risk losing perspective on the clinical relevance of these findings, like the proverbial blind men describing the elephant. Data suggest that the balance between different immune responses over the entire course of infection affects the clinical outcome (Figure 2 bottom). Persistence of memory responses, and the impact of sequential exposures to circulating viruses and vaccines, are important influences on the immunological repertoire at the time of exposure. What factors determine the durability of flavivirus-specific memory after natural infection and vaccination? Which components of memory contribute most to the clinical outcome of subsequent infection? How does the lifetime history of flavivirus exposures affect these associations? Given the co-circulation of multiple flaviviruses, host and viral genetic differences, and variable time intervals, the number of permutations to be considered is enormous. We believe that studies of carefully selected cohorts of subjects with detailed clinical characterization offer critical insights into these questions and should remain a high priority for further study.

Acknowledgements

This work was supported in part by funding from the US National Institutes of Health-National Institute of Allergy and Infectious Diseases (NIH-NIAID) award number P01 AI034533. The views expressed are those of the authors and do not necessarily reflect the official policy or position of the National Institutes of Health.

References

1. Medin CL, Rothman AL: **Zika virus: The agent and its biology, with relevance to pathology**. *Arch Pathol Lab Med* 2017, **141**:33–42.

2. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, et al.: **The global distribution and burden of dengue.** *Nature* 2013, **496**:504–507.

3. CASALS J: **Relationships Among Arthropod-Borne Animal Viruses Determined By Cross-Challenge Tests**. *Am J Trop Med Hyg* 1963, **12**:587–596.

4. Halstead SB: **Observations related to pathogensis of dengue hemorrhagic fever. VI. Hypotheses and discussion.** *Yale J Biol Med* 1970, **42**:350–362.

5. Mongkolsapaya J, Dejnirattisai W, Xu XN, Vasanawathana S, Tangthawornchaikul N, Chairunsri A, Sawasdivorn S, Duangchinda T, Dong T, Rowland-Jones S, et al.: **Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever**. *Nat Med* 2003, **9**:921–927.

6. Friberg H, Bashyam H, Toyosaki-Maeda T, Potts JA, Greenough T, Kalayanarooj S, Gibbons R V., Nisalak A, Srikiatkhachorn A, Green S, et al.: **Cross-reactivity and**

expansion of dengue-specific T cells during acute primary and secondary infections in humans. *Sci Rep* 2011, **1**.

7. Friberg H, Burns L, Woda M, Kalayanarooj S, Endy TP, Stephens HAF, 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**:122–129.

8. Zellweger RM, Shresta S: **Mouse models to study dengue virus immunology and pathogenesis**. *Front Immunol* 2014, **5**.

9. Mathew A: **Humanized mouse models to study human cell-mediated and humoral responses to dengue virus**. *Curr Opin Virol* 2017, **25**:76–80.

10. Chandele A, Sewatanon J, Gunisetty S, Singla M, Onlamoon N, Akondy RS, Kissick HT, Nayak K, Reddy ES, Kalam H, et al.: **Characterization of Human CD8 T Cell Responses in Dengue Virus-Infected Patients from India**. *J Virol* 2016, **90**:11259–11278.

11. Townsley E, Woda M, Thomas SJ, Kalayanarooj S, Gibbons R V., Nisalak A, Srikiatkhachorn A, Green S, Stephens HAF, Rothman AL, et al.: **Distinct activation phenotype of a highly conserved novel HLA-B57-restricted epitope during dengue virus infection**. *Immunology* 2014, **141**:27–38.

12. Haltaufderhyde K, Srikiatkhachorn A, Green S, Macareo L, Park S, Kalayanarooj S, Rothman AL, Mathew A: **Activation of Peripheral T Follicular Helper Cells during Acute Dengue Virus Infection**. *J Infect Dis* 2018, **218**:1675–1685.

* This flow cytometry study demonstrated activation and expansion of circulating T follicular helper cells during acute dengue virus infection and significant associations with

secondary infection and more severe illness.

13. Rivino L, Kumaran E a P, Jovanovic V, Nadua K, Teo EW, Pang SW, Teo GH, Gan VCH, Lye DC, Leo YS, et al.: **Differential targeting of viral components by CD4+ versus CD8+ T lymphocytes in dengue virus infection.** *J Virol* 2013, **87**:2693–706.

14. Rivino L, Kumaran EA, Thein TL, Too CT, Gan VCH, Hanson BJ, Wilder-Smith A, Bertoletti A, Gascoigne NRJ, Lye DC, et al.: **Virus-specific T lymphocytes home to the skin during natural dengue infection**. *Sci Transl Med* 2015, **7**.

15. Katzelnick LC, Gresh L, Halloran ME, Mercado JC, Kuan G, Gordon A, Balmaseda A, Harris E: **Antibody-dependent enhancement of severe dengue disease in humans**. *Science (80-)* 2017, **358**:929–932.

* This study analyzed dengue virus-specific antibody titers from a longitudinal cohort in Nicaragua. Low antibody titers were strongly associated with the risk for dengue hemorrhagic fever, while a weaker association was observed with the more recent classification of severe dengue.

16. Libraty DH, Endy TP, Houng HH, Green S, Kalayanarooj S, Suntayakorn S, Chansiriwongs W, Vaughn DW, Nisalak A, Ennis FA, et al.: **Differing Influences of Virus Burden and Immune Activation on Disease Severity in Secondary Dengue-3 Virus Infections**. *J Infect Dis* 2002, **185**:1213–1221.

17. Zivny J, DeFronzo M, Jarry W, Jameson J, Cruz J, Ennis FA, Rothman AL: **Partial agonist effect influences the CTL response to a heterologous dengue virus serotype**. *J Immunol* 1999, **163**:2754–2760.

18. Zivny J, Kurane I, Ennis FA: **Establishment of dengue virus-specific human CD4+ T lymphocyte clones from Percoll-purified T lymphoblasts by stimulation with monoclonal antibody to CD3**. *J Immunol Methods* 1995, **188**:165–167.

19. Mangada MM, Rothman AL: **Altered Cytokine Responses of Dengue-Specific CD4+ T Cells to Heterologous Serotypes**. *J Immunol* 2005, **175**:2676–2683.

20. Bashyam HS, Green S, Rothman AL: **Dengue virus-reactive CD8+ T cells display quantitative and qualitative differences in their response to variant epitopes of heterologous viral serotypes.** *J Immunol* 2006, **176**:2817–2824.

21. 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**:1282–1291.

22. Friberg H, Beaumier CM, Park S, Pazoles P, Endy TP, Mathew A, Currier JR, Jarman RG, Anderson KB, Hatch S, et al.: **Protective versus pathologic pre-exposure cytokine profiles in dengue virus infection**. *PLoS Negl Trop Dis* 2018, **12**.

** This study measured dengue virus-specific T cell responses in blood samples collected prior to secondary dengue virus infections from a prospective cohort study of Thai children. The analyses identified one set of effector responses associated with subclinical infection and a separate set of responses associated with symptomatic infection.

23. Sánchez-Vargas LA, Kounlavouth S, Smith ML, Anderson KB, Srikiatkhachorn A, Ellison DW, Currier JR, Endy TP, Mathew A, Rothman AL: **Longitudinal analysis of Memory B and T Cell responses to dengue virus in a 5-year prospective cohort study in Thailand**. *Front Immunol* 2019, **10**.

* This study measured the perssitence of dengue virus-specific memory T and B cell responses in sequential blood samples collected from Thai children and analyzed the impact of secondary dengue virus infections on the responses.

24. Calarota SA, Baldanti F: **Enumeration and characterization of human memory t cells by enzyme-linked immunospot assays**. *Clin Dev Immunol* 2013, **2013**.

25. Robinson ML, Durbin AP: **Dengue vaccines: Implications for dengue control**. *Curr Opin Infect Dis* 2017, **30**:449–454.

26. Screaton G, Mongkolsapaya J: **Which dengue vaccine approach is the most promising, and should we be concerned about enhanced disease after vaccination?: The challenges of a dengue vaccine**. *Cold Spring Harb Perspect Biol* 2018, **10**.

27. Angelo MA, Grifoni A, O'Rourke PH, Sidney J, Paul S, Peters B, de Silva AD, Phillips E, Mallal S, Diehl SA, et al.: **Human CD4 + T Cell Responses to an Attenuated Tetravalent Dengue Vaccine Parallel Those Induced by Natural Infection in Magnitude, HLA Restriction, and Antigen Specificity** . *J Virol* 2017, **91**:1–12.

28. Weiskopf D, Angelo MA, Bangs DJ, Sidney J, Paul S, Peters B, de Silva AD, Lindow JC, Diehl SA, Whitehead S, et al.: **The Human CD8 + T Cell Responses Induced by a Live Attenuated Tetravalent Dengue Vaccine Are Directed against Highly Conserved Epitopes** . *J Virol* 2015, **89**:120–128.

29. Friberg H, Martinez LJ, Lin L, Blaylock JM, De La Barrera RA, Rothman AL, Putnak JR, Eckels KH, Thomas SJ, Jarman RG, et al.: **Cell-Mediated Immunity Generated in Response to a Purified Inactivated Vaccine for Dengue Virus Type 1**. *mSphere* 2020, **5**.

30. Waickman AT, Victor K, Li T, Hatch K, Rutvisuttinunt W, Medin C, Gabriel B, Jarman RG, Friberg H, Currier JR: **Dissecting the heterogeneity of DENV vaccine-elicited cellular immunity using single-cell RNA sequencing and metabolic profiling**. *Nat Commun* 2019, **10**:1–16.

** This study applied single-cell RNA sequencing and T cell receptor sequencing to sequential blood samples collected from recipients of a tetravalent live attenuated dengue vaccine. Significant changes in the expression of metabolic gene pathways were identified in memory precursor T cells during the acute phase of the immune responses which were further validated using flow cytometry.

Figure legends

Figure 1. Illustrative examples of DENV-specific T cell responses in cohort studies. A. Longitudinal analysis of DENV-specific T cells in a prospective cohort of Thai children. Frequencies of IFN-γ spot-forming cells (SFC) detected in cultured ELISPOT assays after restimulation with peptide pools from DENV-2 structural (2prM/E) or non-structural (2NSA) proteins using PBMC collected at the indicated time points; subjects experienced a secondary DENV-2 infection in 2001. NT, not tested. B. Analysis of DENV-specific T cell responses to vaccination in a Philippine cohort. Frequencies of IFN-γ SFC detected in ex vivo ELISPOT assays after stimulation with peptide pools from structural (prM/E) or non-structural (NSA) proteins of each DENV serotype or NS3 protein of YFV using PBMC collected prior to vaccination (V01), 1 month after the first vaccination (V02), or 1 month after the third vaccination.

Figure 2. Model of T cell involvement in dengue disease. A) Different subsets of T cells are induced during DENV infections. In a primary DENV infection, serotype-specific T cells are activated including cytotoxic T cells, helper T cells comprising the recent described T_{FH} cells & Tregs. In the course of convalescent-phase, T_{EM} & T_{CM} are the long-term memory subsets. These memory T cell pools are modified by a second flavivirus infection or vaccination resulting in the expansion of cross-reactive and recently activated serotype-specific clones. B) Multiple factors associated with disease severity. Factors listed on the left side are hypothesized to contribute to enhanced disease severity; factors listed on the right are hypothesized to contribute to protection from infection and/or illness.