



# Understanding the Human T Cell Response to Dengue Virus

# 17

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## Abstract

Our understanding of how T cells respond to dengue virus has greatly advanced in the last decade but important questions still remain unanswered. Dengue virus infection elicits a broad anti-viral T cell response with NS3, NS4b and NS5 being the main targets for CD8+ T cells, which dominate the response while the structural proteins capsid, envelope and the secreted protein NS1 are the preferential targets for CD4+ T cells. Upon T cell activation during acute dengue infection, dengue-specific T cells acquire expression of the skin-homing marker cutaneous associated antigen (CLA) and they can be found at high frequencies in the skin of infected patients. This suggests that the skin represents an important site for the immuno surveillance of dengue virus. The immunoprotective role of skin-homing dengue-specific T cells, their potential involvement in pathological skin manifestations and their long-term persistence as tissue resident T cells to provide immediate onsite protection are open questions that we are currently investigating. The contribution of pre-existing dengue-specific T cells towards protective immunity and/or immunopathology

during secondary dengue infection remains a major knowledge gap. The evidence supporting these opposing outcomes and our current understanding of the characteristics of the human T cell response to dengue virus will be discussed.

## Keywords

CD4+ and CD8+ T cells · Original antigenic sin · Skin homing marker · Dengue T cells · Tissue-resident T cells

## 17.1 Role of T Cell Immunity and “Original Antigenic Sin”

CD4+ and CD8+ T cells are an essential component of protective immunity against viral infections and understanding their role and how they develop is critical for the design of optimal vaccines.

Acute viral infections resulting in viral clearance generally elicit effective anti-viral T cell responses that progress through three phases: a period of activation and expansion of virus-specific T cells, a contraction phase where the majority of effector cells undergo death followed by the establishment and maintenance of a pool of virus-specific memory T cells [35]. These memory T cells, together with other components

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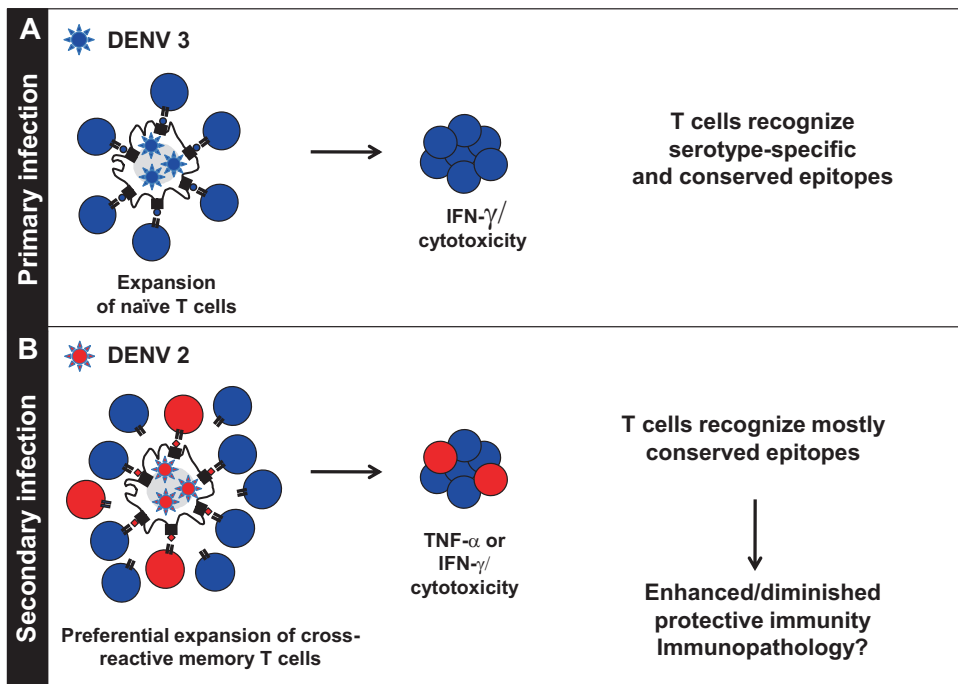
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of the immune system, will confer protection upon re-infection with the same virus. In particular, CD8+ T cells are important for viral clearance as they can directly lyse virus-infected cells through the production of IFN- $\gamma$  and cytotoxic molecules such as perforin and granzymes. These processes involve the recognition through the T cell receptor (TCR) of viral peptides of 9–10 aminoacids in length, that are presented on the cell surface of antigen presenting cells and virus-infected cells in association with molecules of the human leukocyte antigen (HLA) system. CD4+ T cells recognize longer peptides of 12–15 aminoacids in length and have a more diverse function as they are required to develop a broad and efficient antibody response and they are critical for the generation of both B cell and CD8+ T cell memory responses. In addition, CD4+ T cells can also directly kill virus-infected cells through production of IFN- $\gamma$  and cytotoxic effector functions in a similar manner to CD8+ T cells.

Successful vaccines such as the vaccinia virus vaccine (which led to the eradication of small pox) and the yellow fever vaccine are known to elicit strong and long-lasting antibody and T cell responses [17, 23]. We believe that the induction of both components of the immune system is key to a successful vaccine. However, designing a vaccine for dengue virus has proved to be particularly challenging because of the co-circulation of four distinct serotypes that share approximately 70% of sequence homology. Thus, individuals living in a dengue-endemic region will be exposed to multiple dengue serotypes during their lifetime. Infection with one dengue serotype will confer life-long protective immunity towards the same serotype but evidence shows that that it increases the risk of DHF/DSS upon secondary infection with another serotype [11]. An explanation for these observations was provided by a mechanism called “antibody dependant enhancement” (ADE) whereby anti-DENV antibodies that are generated during a primary dengue infection will bind to – but poorly neutralize – the secondary infecting serotype due to variations in the peptide sequence between the two viruses.

Suboptimal neutralization results in increased uptake of virus-antibody complexes through the Fc $\gamma$ R present on antigen presenting cells with subsequent increased viral load and antigen-presentation and enhanced immune activation. In 2003 the group of Gavin Screaton proposed a similar mechanism for T cells whereby during secondary dengue infections the T cell response is dominated by weakly cross-reactive memory T cells specific for the primary infecting virus that have suboptimal anti-viral capacity towards the secondary infecting serotype but secrete pro-inflammatory cytokines such as TNF- $\alpha$ , which contribute to plasma leakage [18]. This process known as “original antigenic sin” was first described in a mice to impair CD8+ T cell recognition and clearance of a secondary-infecting Lymphocytic Choriomeningitis Virus (LCMV) bearing mutations in T cell epitopes [13]. However, subsequent mouse models showed that the presence of cross-reactive T cells could in some cases be beneficial. For example, pre-existing memory cells that are cross-reactive with vaccinia virus can protect mice from an otherwise lethal dose of vaccinia virus [4]. Different mouse models of secondary virus infection have shown that pre-existing cross-reactive T cell immunity can alter the hierarchy of the T cell response to a secondary-infecting virus leading to enhanced or diminished protective immunity and altered immunopathology. There is also evidence of a strong skewing of the T cell repertoire in individuals with secondary DENV infections compared to those with primary infection or in vaccinees receiving a tetravalent live-attenuated dengue vaccine as opposed to the monovalent vaccine [32, 33]. However, the implications of the skewed T cell repertoire for T cell functionality remain unclear (Fig. 17.1).

At a molecular level, because the TCR interacts with only few aminoacids within the 9–10 mer peptide, it can accommodate aminoacid variations providing they do not disrupt the peptide-TCR or the peptide-HLA interactions. However, the result of this variation in terms of avidity of the T cell for the peptide and level of T cell



**Fig. 17.1** Schematic depicting the process of “original antigenic sin” for T cells during dengue infection and its possible implications for protective immunity and/or immunopathology. (a) During a primary infection for example with DENV 3, naïve T cells with high avidity for DENV 3-derived peptides undergo expansion. The resulting dengue-specific T cell pool will comprise of T cells specific for peptides that are unique to DENV 3 (serotype-specific) and those that are shared amongst 2 or more serotypes (conserved). T cells that have high avidity for their cognate antigen undergo optimal TCR triggering which mainly leads to production of IFN- $\gamma$  and efficient anti-viral function. (b) Upon secondary infection for example with DENV 2, pre-existing T cells generated during the primary DENV 3 infection that are cross-reactive to DENV 2 peptides will preferentially expand compared to their naïve counterpart. This occurs because dengue-specific memory cells are present in

higher frequencies and have a lower activation threshold compared to their naïve counterparts. As a result, the responding T cell pool will be dominated by cross-reactive T cells specific for conserved epitopes. Some cross-reactive T cells may have a lower avidity for DENV 2 antigens which results in suboptimal TCR triggering and subsequent production of high levels of TNF- $\alpha$  and poor anti-viral efficacy with a possible contribution to immunopathology. However, variable proportions of cross-reactive memory T cells may have high avidity for DENV 2 antigens, will undergo optimal TCR triggering resulting in high levels of IFN- $\gamma$  and anti-viral capacity. The outcome of the activation of cross-reactive T cells is therefore difficult to predict and could lead to faster and more efficient protective responses or to different degrees of immunopathology. Both scenarios however should result in skewing of the T cell response towards more conserved epitopes

activation is highly variable and difficult to predict. We believe that the complexity of T cell cross-reactivity is at the basis of the contrasting findings reported over the last decade that point to either a protective or a pathologic role of dengue-specific T cells during secondary dengue virus infections [22]. Several studies support the hypotheses proposed in the 2003 study [18], for example dengue-specific T cells are found to be present at higher frequencies in patients with

DHF as compared to those with DF [6] and in vitro stimulation of T cells with homologous versus heterologous dengue peptides results in cytokine profiles that are both qualitatively and quantitatively distinct [9, 16, 19]. However, a number of other studies do not confirm these findings [8, 10, 14, 29, 30] and there is now accumulating evidence supporting a protective role of T cell immunity during DENV infection, including data from immune-deficient mouse models

[21, 36, 37]. A prospective study in school children shows that asymptomatic secondary dengue infections correlate with increased levels of DENV-specific T cells compared to symptomatic infection<sup>22</sup>. In healthy adults from dengue endemic regions HLA class I and II molecules associated with decreased susceptibility to severe dengue disease were shown to support memory T cell responses of higher magnitude, suggesting a protective rather than a detrimental role of T cells [31, 34]. Further studies that directly compare the primary versus secondary T cell response to individual DENV serotypes are urgently needed to clarify the role of these cells during infection and to reconcile the contrasting findings reported in the literature. The complex scenario that emerges from the above studies further highlights that it is mandatory for a dengue vaccine to elicit a balanced T cell (as well as antibody) response towards all four DENV serotypes.

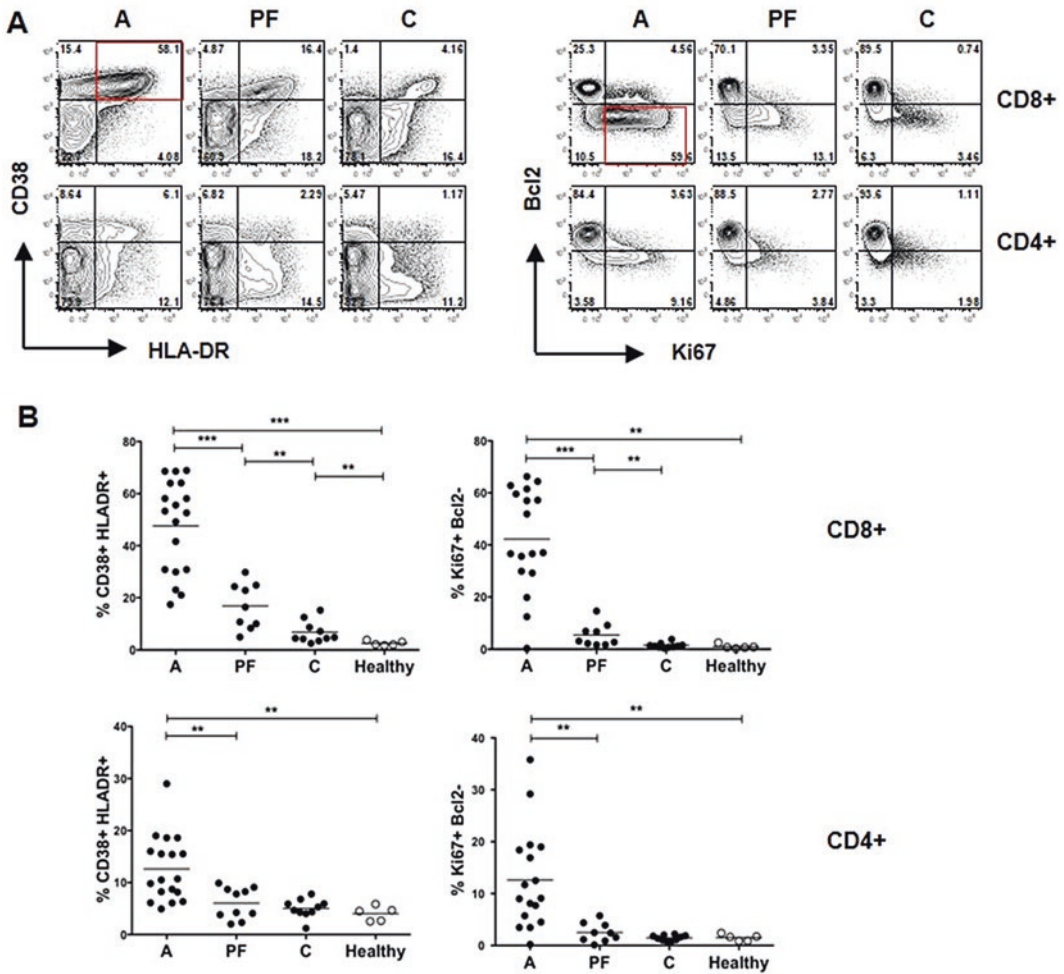
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## 17.2 T Cell Responses during Dengue Virus Infection

To better understand the role of T cells during dengue infection we sought to characterize the nature of the T cell response during the course of dengue infection, from acute infection to convalescence. Dengue patients were recruited by the team of Prof Leo Yee Sin at CDC Tan Tock Seng Hospital in Singapore and blood was drawn from the patients at three different time points after diagnosis of dengue infection (days from fever onset: acute day 6–9, post-febrile day 14–21 and convalescent day 60–120). Peripheral blood mononuclear cells (PBMCs) were analyzed at all time points. The first striking observation that we made was that acute dengue infection induces a massive activation and proliferation of CD8+ T cells and a significant but more modest activation and expansion of CD4+ T cells. Activated (HLA-DR+ CD38+) and proliferating (Ki67+ Bcl2-) T cells are found at highest frequencies during acute dengue and represent in some patients up to 70% of all CD8+ T cells (high-

lighted with a red box in Fig. 17.2a). The proportion of activated and proliferating T cells decreases in the post-febrile and convalescent phases of disease reaching levels comparable to those found in healthy individuals. Representative flow cytometry profiles of the activation and proliferation of CD4+ and CD8+ T cells from a dengue patient are shown in Fig. 17.2a and results from 18 patients are summarized in Fig. 17.2b. Given the large numbers of activated CD8+ T cells observed in the blood of acute dengue patients, it is likely that a proportion of these cells will be bystander-activated and will thus not be specific for DENV. We and others have shown that acute dengue infection is accompanied by the activation and proliferation of CD8+ T cells that are specific for persistent viruses such as human cytomegalovirus or Epstein-Barr virus [25, 26]. These cells may be activated *in vivo* in a TCR-independent manner through the action of cytokines such as IL-15 that are abundantly produced upon viral infection [26]. However the exact enumeration of dengue-specific T cells is challenging as we rely on functional assays such as IFN- $\gamma$  production after DENV peptide stimulation which are unable to detect T cells with impaired cytokine-producing capacities and are thus likely to underestimate the number of dengue-specific T cells.

Our knowledge of the kinetics and the protein targets of the dengue-specific T cell response during acute dengue infection has greatly advanced over the last decade. We know that dengue infection elicits a DENV-specific T cell response that peaks around days 8–10 from fever onset [6, 24, 29]. The T cell response is broad as it targets all viral proteins to some extent, with a preferential recognition of the non-structural proteins NS3, NS4b and NS5. Similarly to what occurs in other acute viral infections dengue-specific CD8+ T cells are present at higher frequencies compared to CD4+ T cells. While CD8+ T cell responses preferentially target the non-structural proteins NS3 and NS5, CD4+ T cells mainly target the structural proteins capsid and envelope and the secreted proteins NS1, consistent with virions being the



**Fig. 17.2** High frequencies of activated and proliferating T cells during acute dengue infection. PBMC samples from dengue patients are analyzed during the acute (A), post-febrile and (PF) convalescent (C) phases of disease. Live cells are surface stained with antibodies against CD3, CD4, CD8, CD38 and HLA-DR followed by intracellular staining for Ki67 and Bcl2. Isotype controls are used to define negativity for CD38, HLA-DR, Ki67 and Bcl2. (a) Activation and proliferation profiles of CD8+

(top panel) and CD4+ T cells (bottom panel) for one representative patient. Activated and proliferating T cells are defined as CD38+ HLA-DR+ and Ki67+ Bcl2- cells, respectively (see red box for CD8+ T cells). (b) Results from 18 patients are summarized for CD8+ (top panel) and CD4+ T cells (bottom panel). Statistics are calculated by Mann-Whitney non-parametric test. Values are considered significant when  $p < 0.05$

main source of antigen for CD4+ T cells [24]. More recent studies have reported that these patterns of immunodominance vary as a function of the infecting serotype with DENV 3 eliciting CD8+ T cells that target both structural and non-structural proteins while DENV 1, 2 and 4 elicit CD8+ T cells targeting mainly NS3, NS4b and NS5 [32, 33].

### 17.3 Phenotype and Tissue-Homing of Dengue-Specific T Cells

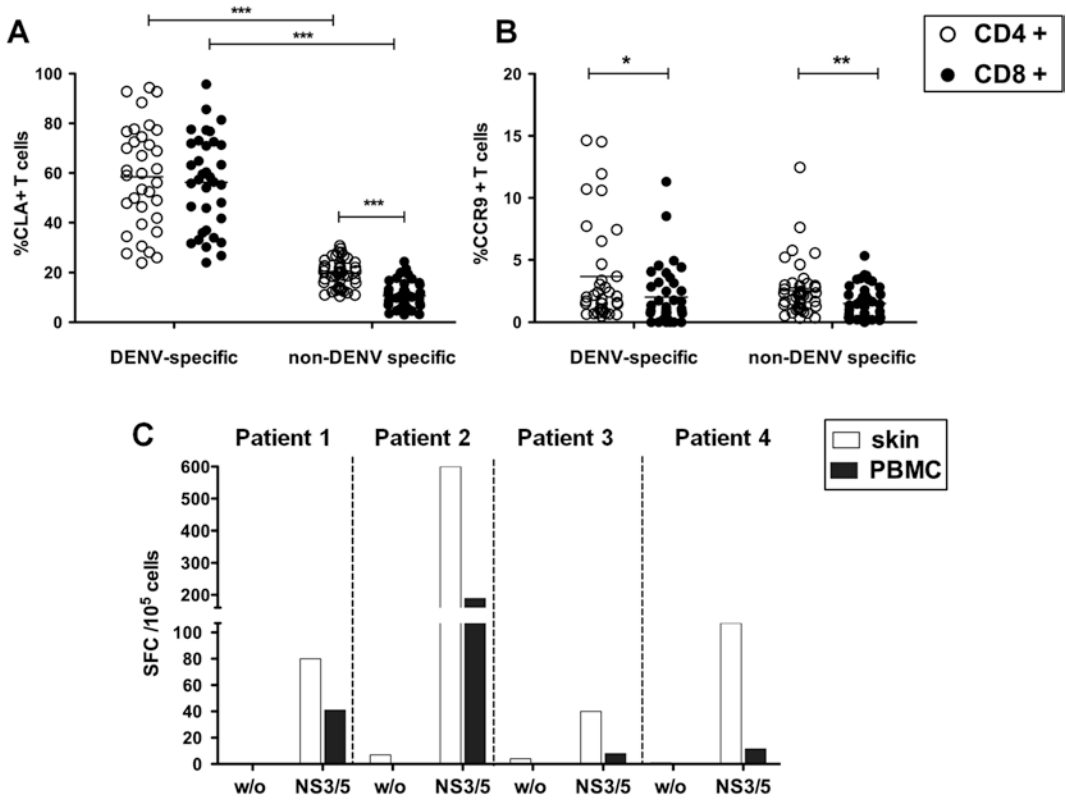
The identification of a large number of T cell epitopes, the majority of which are CD8+ T cell epitopes, has provided us with tools to better characterize T cells during dengue infection or vaccination. As of September 2017 1613 immu-



nogenic peptides have been deposited in the Immune Epitope Database (<http://www.iedb.org/>). In particular, the study of CD8+ T cells has been facilitated by the use of peptide-HLA tetramers which are streptavidin-labelled MHC multimers bound to the peptide of interest. These tools have revolutionized the way we can look at T cell immunity as peptide-specific CD8+ T cells can be enumerated and characterized phenotypically directly *ex vivo* by flow cytometry without relying on a functional read-out [1]. However, the definition of immunodominant epitopes restricted to HLA molecules that are commonly expressed in populations from dengue-endemic regions remains elusive and the selection of T cell epitopes to use for peptide-HLA tetramer analyses of dengue-infected individuals can still be a difficult task.

By using peptide-HLA tetramers for the previously described HLA-A\*1101-restricted dengue NS3 27 epitope [18] we addressed the functional and phenotypic characteristics of dengue-specific T cells during the course of dengue infection. We show that during secondary acute dengue infection NS3-27-specific T cells are highly activated and proliferating, produce IFN- $\gamma$  but not TNF- $\gamma$  and are able to lyse dengue-infected cells, suggesting that at least *in vitro* their anti-viral function does not appear to be impaired [25]. To address the capacity of dengue-specific T cells to migrate to different organs and to potentially contribute to immunopathology, we studied the homing receptors present on the surface of these cells. During acute dengue infection NS3 27-specific CD8+ T cells express the skin homing molecule cutaneous lymphocyte-associated antigen (CLA), the chemokine receptors CXCR3 and CCR5 which allow migration to inflamed tissues, but not the gut-homing receptor CCR9. Expression of CLA but not of CCR9 was confirmed for the total population of CD4+ and CD8+ T cells specific for NS3 and NS5 (Fig. 17.3a, b). We show that expression of the skin-homing marker CLA by dengue-specific T cells correlates with their presence in the skin tissue where they could be detected at higher frequencies as compared to peripheral blood [25] (Fig. 17.3c). The exact role of dengue-specific T

cells in the skin during acute infection, whether they persist long-term in this site as tissue-resident memory T cells upon resolution of the infection and their possible protective role upon secondary infections is currently under investigation. The expression of CLA on dengue-specific T cells during acute dengue is consistent with their initial activation by skin-derived dendritic cells. Dengue virus enters the body through the skin during the blood meal of an infected mosquito, infects local dendritic cells [3, 28] and travels to skin-draining lymph-nodes where it interacts with virus-specific T cells. Studies have shown that during activation T cells acquire the capacity to migrate to certain tissues by expressing different sets of receptors that serve as “address-codes”, the expression of which is determined by the antigen presenting cell and by the local microenvironment [7]. It was shown for example that during T cell priming, tissue-derived antigen presenting cells within cutaneous versus intestinal secondary lymphoid organs imprint the corresponding tissue-specific homing phenotype to the T cell, such that the primed T cells have a predisposition to home back to the skin or gut, respectively [2, 20]. More recently, studies have shown that the route of immunization strongly impacts the migratory capacity and consequently the protective efficacy of T cells upon re-infection, as T cells need to localize to the sites of virus replication in order to exert their function [15, 27]. For example, vaccination with live vaccinia virus was 10<sup>5</sup> times more effective in protecting against viral re-infection if delivered by skin scarification as compared to subcutaneous, intradermal, and intramuscular vaccination [12]. The authors show that immunization by skin scarification was associated with local keratinocyte infection and generation of a long-lived tissue resident CD8+ T cell memory populations. The presence of tissue resident CD8+ T cells was sufficient to protect against re-infection with vaccinia virus and did not require antibodies or recruitment of circulating blood T cells [15]. These considerations highlight an important aspect of T cell immunity that is often not considered in the context of vaccination. We now know that pathogen infection generates



**Fig. 17.3** Dengue-specific T cells have skin-tissue migratory properties during acute dengue infection. Expression of the skin-homing receptor CLA (a) and the gut-homing receptor CCR9 (b) is assessed by flow cytometry on dengue-specific T cells from the peripheral blood of acute dengue patients. PBMCs are briefly stimulated with or without NS3 and NS5 peptides and live cells are stained for expression of CD3, CD4, CD8, CLA and CCR9 followed by intracellular cytokine staining for IFN- $\gamma$  and TNF- $\alpha$ . Expression of CLA and CCR9 is analysed on cytokine-producing CD4+ and CD8+ T cells (defined as “DENV-specific”) or on those that lack cytokine production (defined “non-DENV specific”). The percentage of CLA+ or CCR9+ cells is shown for CD4+

(white circles) and CD8+ T cells (black circles). Isotype controls are used to define negativity for CLA and CCR9 expression. (c) The presence of dengue-specific T cells in the skin was investigated by raising skin suction blisters on the forearm of dengue patients. PBMCs or cells obtained from skin suction blisters are stimulated with or without NS3 and NS5 peptides and analysed for production of IFN- $\gamma$  by ELISPOT. Results obtained from cells derived from the skin (white bar) or the peripheral blood (black bar) are expressed as Spot Forming Cells relative to 10<sup>5</sup> cells (SFC/10<sup>5</sup>). Statistics were calculated using a Kruskal-Wallis test, followed by a non-parametric Mann-Whitney test. (Adapted from Rivino et al. *Sci Trans Med* [25])

populations of non-recirculating tissue-resident memory T cells that persist long-term within tissues such as the skin, lung, gastro-intestinal tract and reproductive tract. These cells have potent effector functions and provide rapid on-site protection in peripheral tissues upon re-infection with previously-encountered pathogens [5]. A better understanding of the localization of dengue-specific memory T cells in dengue-

immune individuals and how these cells can be generated in the context of vaccination would greatly aid the design of dengue vaccines with improved protective efficacy.

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## Discussion of Chapter 17 in Dengue and Zika: Control and Antiviral Treatment Strategies

*This discussion was held at the 2nd Advanced Study Week on Emerging Viral Diseases at Praia do Tofo, Mozambique.*

*Transcribed by Hilgenfeld R and Vasudevan SG (Eds); approved by Dr. Laura Rivino.*

**Anuja Mathew:** So you found higher frequencies of CD8+ T cells in the skin. Do you have an hypothesis of why you would see a preference for CD8+ or CD4+ T cells?

**Laura Rivino:** We have preliminary data showing that the dengue-specific T cells that we find in the skin are mostly CD8+ T cells, so we think these cells could account in part for the increase of total CD8+ T cells that we find during acute dengue in the skin. We do not expect this influx of CD8+ T cells to comprise of only dengue-specific T cells. We know, for example from studies performed by us and others that in the blood of acute dengue patients there is also “bystander-activation” of CD8+ T cells that are specific for non-related, persistent viruses such as HCMV and EBV. So the influx of CD8+ T cells that occurs in the skin of acute dengue patients could comprise of both dengue-specific and “bystander-activated” T cells.

This higher frequencies of CD8+ T cells that we observe in the skin of acute dengue patients was not surprising to us since for other skin viral infections, such as Herpes simplex virus, the anti-viral response at the skin level is mainly a CD8+ T cell response.

**Katja Fink:** The cytokines that you find in the blister fluid are more or less the same as what is observed in the plasma or in the skin?

**Laura Rivino:** No. So the increase in pro-inflammatory cytokines is more pronounced in the skin compared to the blood. We have done quite a lot of cytokine profiling from the plasma of dengue patients and the increase in pro-inflammatory cytokines at the time points we look at are obvious only for the more severe dengue cases. We generally do not see

significant increases of these cytokines in the blood, especially of TNF- $\alpha$ .

**Katja Fink:** So you compared skin and blood samples from the same patients?

**Laura Rivino:** Yes.

**Aravinda da Silva:** Were these cases primary infections?

**Laura Rivino:** Most of our patients are experiencing secondary infections. We had few primary infections so it’s difficult to really make a statement, but when I looked separately at patients with primary and secondary infection I didn’t see a very big difference in terms of immunodominance of the different dengue proteins. The immunodominance was similar in primary and secondary cases and also for different dengue serotypes as well.

**Aravinda da Silva:** Right, but in terms of seeing the cells so early in the skin by day four of onset of symptoms, it seems very fast for a primary response. Do you see it that fast and in those numbers even in primary infection?

**Laura Rivino:** We don’t have the data yet of which patients were experiencing primary or secondary infection for the skin samples.

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