Analysis of cell-mediated immune responses in support of dengue vaccine development efforts

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ABSTRACT

Dengue vaccine development has made significant strides, but a better understanding of how vaccine-induced immune responses correlate with vaccine efficacy can greatly accelerate development, testing, and deployment as well as ameliorate potential risks and safety concerns. Advances in basic immunology knowledge and techniques have already improved our understanding of cell-mediated immunity of natural dengue virus infection and vaccination. We conclude that the evidence base is adequate to argue for inclusion of assessments of cell-mediated immunity as part of clinical trials of dengue vaccines, although further research to identify useful correlates of protective immunity is needed.

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1. Introduction

Q2 The immunological basis of the efficacy of many of the most well-established vaccines is poorly understood, and, where studies to better understand vaccine efficacy have been done, they have almost always relied on tests of pathogen-specific antibodies rather than on measures of cell-mediated immunity (CMI) [1]. Several reasons likely explain this bias; serum is more easily obtained than viable lymphocytes, antibodies can be studied in isolation, and assays of antibody concentration and function are technically more straightforward and reproducible than cellular assays. Fortunately, in many cases detection of antibodies at or above a defined concentration using specific assays has proven to serve as a useful correlate of protective immunity. However, there has been ample evidence in the case of established vaccines that the information provided by assays of antibody responses is often incomplete, and that protective immunity (sometimes only partially protective) was present in some individuals without protective antibody levels.

A consultation was organized by the WHO in 2007 to "review the state of the art of dengue CMI and to discuss the potential role of CMI in advancing dengue vaccine candidates towards licensure" [2]. The participants concluded that "precise function of CMI in protection or disease pathology remains ill-defined and, at present, there is no evidence to suggest that CMI can be utilized as a correlate of protection." Recent data from dengue vaccine trials has renewed interest in addressing this issue, however. In the pivotal phase III trials of the Sanofi Pasteur chimeric dengue virus (DENV) – yellow fever virus (YFV) vaccine, plaque reduction neutralization titers (PRNT) only weakly correlated with protection, and breakthrough infections occurred in some individuals with high PRNT values [3,4]. While efforts continue to refine assays of DENV-specific antibodies in order to discriminate effective/protective from ineffective/non-protective antibodies (assuming that this is possible), these findings re-emphasize the need to consider the role of DENV-specific T lymphocyte responses in vaccine efficacy. This review seeks to summarize the current state of knowledge regarding DENV-specific CMI and propose potential contributions of CMI measurements to dengue vaccine development and testing.

An appraisal of the literature on DENV-specific T cell responses merits a brief review of current paradigms in T cell biology and relevant technologies. One area highlighted by recent work is the complexity of effector T cell subsets. Extending the paradigm of Th1 versus Th2 responses among CD4 T cells, at least seven different phenotypes have now been described [5,6]. Table 1 summarizes key proteins expressed by each subset. Cytokines and other signals produced by antigen-presenting cells during the initial T cell activation (not listed in the table) determine which pathway is taken by an individual T cell through the induction of the transcription factors listed, and this in turn controls the profile of chemokine receptors and cytokines produced. The characteristic cytokines produced by each subset are the major determinant of its role in immunity and also tend to reinforce cell polarization. The profile of chemokine

Table 1
Characteristics defining different subsets of effector CD4 T cells.

Subset	Cytokine(s) produced	Chemokine receptor(s)	Transcription factor(s)	Comment
Th1	IFN-γ	CXCR3	T-Bet	Cellular immunity
Th2	IL-4, IL-5, IL-13	CCR3, CCR4, CCR8	GATA-3	Humoral immunity
Th17	IL-17	CCR2, CCR4, CCR6	RORγt	Inflammation
Th9	IL-9	CCR3, CCR6, CXCR3	PU.1	Mucosal immunity
Th22	IL-22	CCR4, CCR10	AhR	Parasites
Tfh	IL-21	CXCR5	Bcl-6	B cell help
iTreg	IL-10, TGF-β	CCR6	FoxP3	Immunosuppression, tolerance

receptors expressed by each cell subset determines that subset's predominant anatomical distribution, such as peripheral versus mucosal versus secondary lymphatic sites, which also contributes to its function in the response to different pathogens. Cytolytic activity, not traditionally considered an important effector function of CD4 T cells, has been increasingly recognized, mainly among cells expressing Th1 cytokines [7]. In contrast, while cytolysis has long been seen as the main function of CD8 T cells, there has been a growing recognition of more diverse subsets within this population. CD8 T cell subsets with cytokine profiles similar to several of the CD4 subsets listed in Table 1 have been described, although there is comparably less known about them. Based on studies in mice, T cell polarization has often appeared to be a fixed characteristic of the cell determined during its initial activation. However, studies in humans suggest more plasticity in T cell phenotype [8].

Another area of active research in T cell biology is the developmental relationships between naïve, effector, and memory T cells [9–11]. This topic entails significant debate, as, unlike the case with B lymphocytes, there are no universally accepted standards for defining a memory T cell; several different schemas have been proposed to define the phenotypes of effector versus memory T cells, but it is clear that these are imperfect. From a functional standpoint, it is recognized that, among antigen-experienced T cells, there is a subset of short-lived effector cells that are destined to undergo apoptosis whereas other cells demonstrate the capacity for long-term persistence and even self-renewal. Within the long-lived memory cell population, heterogeneity in function and protein expression led to a distinction of central memory T cells (T_{CM}) and effector memory T cells (T_{EM}). Recent data have revealed further complexity, and led to the classification of several additional subsets such as tissue-resident memory T cells (T_{RM}) and stem memory T cells. Rather than fixed cell fates, however, there is evidence that these phenotypes retain some degree of plasticity. The timing and determinants of the transitions between states are not fully understood, and remain an important area of investigation. Several markers have been clearly identified as strongly associated with a cell's capacity for long-term survival, such as high expression of IL-7R and low expression of KLRG1.

2. Assay methods

Persisting antibody following vaccination is recognized as the first line of defense against subsequent infection and is regarded as a distinguishing characteristic of an effective vaccine [1]. All currently licensed anti-viral vaccines elicit a robust antibody response that correlates with the level of protection provided by the vaccine [12]. If the same should prove to be true for dengue, then the search for a CMI "correlate of protection" for dengue would be unnecessary. However, dengue is one of several globally important infectious diseases, along with HIV, malaria, and tuberculosis, for which a vaccine is highly desirable yet no validated animal model or correlate of immune protection is known. While empirical testing of candidate vaccines has been successful in the past, the era of molecular biology has led to an explosion of tools and methodologies for creating new vaccine antigens and vector delivery systems.

The contribution of CMI, particularly T cells, to a successful dengue vaccine is highly likely whether it be as direct effector cells, provision of help for antibody development or creating a generalized anti-viral environment. Together with the antigenic complexity of candidate dengue vaccines (Table 2), assessing T cell responses presents a logistical problem for both vaccine developers and clinical testing laboratories – how to test or screen for all possible T cell functions when the most relevant function(s) are unknown.

Fortunately, T cell-based immunoassay development has also proceeded at a remarkable rate [13,14]. A list of assays together with their advantages and disadvantages is presented in Table 3. Recently the focus of immune-monitoring has been upon assays that provide "minimal manipulation." Relatively high-throughput assays such as ELISPOT and intracellular cytokine staining (ICS), which utilize in vitro stimulation times of less than 24 h (or no stimulation in the case of direct ex vivo flow cytometry), are the assays of choice as a screening tool. When well qualified, both platforms are quantitative and specific for the antigen. While validation of ELISPOT and ICS assays is not trivial, it is possible, and if a T cell-based correlate of protection for dengue is defined one of these platforms would most likely be the basis of such an assay [15,16]. The general disadvantage of ELISPOT assays is that some a priori knowledge of the relevant functions is required. IFN-γ has been used extensively in vaccine development as a marker of vaccine take and as a function that is necessary, but perhaps not sufficient, for protection. ICS expands upon the functional profile of ELISPOT assays, bringing the concept of polyfunctionality of T cells to the fore. Again, some a priori knowledge of the relevant functional profile is required to fully interpret the results of this assay. Furthermore, ELISPOT and ICS assays are best suited for measuring and quantifying the direct effector capacity of T cells (IFN-γ, TNFα, and cytolytic potential), but are significantly less sensitive at measuring T cell helper capacity. Mass cytometry and advanced polychromatic flow cytometry are technologies that permit the analysis of as many as 36 parameters simultaneously on a single cell. These parameters may include both phenotypic and functional markers. While these methods will facilitate high-dimensional, quantitative analysis of biomolecules on cell populations at single-cell resolution, their application to dengue research has so far been limited [17,18].

The most sensitive assays are generally those that involve proliferation of a small number of antigen-specific precursor cells.

Table 2
T cell antigenic content of dengue vaccine candidates in clinical development.

Vaccine developer	Structural proteins	Non-structural proteins
Live, attenuated (chimeric flaviviruses)		
Sanofi Pasteur	C: YFV; pre-M, E: DENV1-4	NS1-5: YFV
Takeda	C: DENV2; pre-M, E: DENV1-4	NS1-5: DENV2
NIH/Butantan	C: DENV1/3/4; pre-M, E: DENV1-4	NS1-5: DENV1/3/4
Purified inactivated		
WRAIR/GSK	C, pre-M, E: DENV1-4	None (? NS1)
Subunit		
Merck	E (80%): DENV1-4	None