EBioMedicine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Plasmablasts During Acute Dengue Infection Represent a Small Subset of a Broader Virus-specific Memory B Cell Pool

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ARTICLE INFO

Article history:
Received 15 July 2016
Received in revised form 25 August 2016
Accepted 6 September 2016
Available online xxxx

Keywords:
Dengue
Plasmablasts
Memory B cells
Antibodies

ABSTRACT

Dengue is endemic in tropical countries worldwide and the four dengue virus serotypes often co-circulate. Infection with one serotype results in high titers of cross-reactive antibodies produced by plasmablasts, protecting temporarily against all serotypes, but impairing protective immunity in subsequent infections. To understand the development of these plasmablasts, we analyzed virus-specific B cell properties in patients during acute disease and at convalescence. Plasmablasts were unrelated to classical memory cells expanding in the blood during early recovery. We propose that only a small subset of memory B cells is activated as plasmablasts during repeat infection and that plasmablast responses are not representative of the memory B cell repertoire after dengue infection.

1. Introduction

With 390 million people infected every year, dengue is now a global concern [1]. Over the past decades, the virus has spread from South East Asia to regions across the world with climates favorable for breeding of the transmitting vector, the *Aedes* or "tiger" mosquito. In Singapore, where this study was conducted, dengue is endemic and approximately half the adult population is seropositive, providing an excellent opportunity to compare primary and secondary (memory) responses. The dengue virus (DENV) complex comprises four antigenically related viruses (DENV-1 to 4) from the flavivirus family, and infection with one serotype generates both serotype-specific and cross-reactive antibodies [2]. During heterotypic re-infection, the antibody response is dominated by cross-reactive antibodies binding to regions in the viral proteins that are conserved across all serotypes [3,4]. At the same time, neutralizing antibodies against the serotype of the previous infection are often amplified more efficiently than antibodies against the new infecting serotype, which can result in increased disease severity when an individual is re-infected with a different serotype, a phenomenon previously described as original antigenic sin [5,6].

B cell activation, including the activation of pre-existing memory B cells (MBC), contributes to a substantial plasmablast response during acute heterologous infection [7–9], resulting in a high increase in neutralizing antibody titers [10] that contribute to temporary cross-protection against all four serotypes. Recently, we demonstrated that this plasmablast response is polyclonal, but all antibodies cloned from the genes of individual plasmablasts recognized the envelope (E) glycoprotein. In contrast, the majority of previously reported DENV-specific MBCs isolated from the blood of recovered dengue patients were specific to either prM, a membrane protein expressed on immature, non-infectious virus particles, or to non-structural proteins, notably NS1 [11–14], potentially indicating separate pathways of development between plasmablasts and classical MBCs.

The establishment of multiple levels of B cell memory has been suggested previously in mice. It was observed that IgM^+ germinal center (GC) derived MBCs re-entered GC reactions upon re-infection, whereas IgG^+ GC-derived MBCs almost exclusive differentiated into plasmablast [15]. Another elegant study in wild-type mice documented the generation of two distinct memory populations after immunization with the model antigen phycoerythrin: a long-lasting IgM memory population and a more short-lived IgG memory population. Upon re-immunization, switched memory cells differentiated into plasmablasts and proliferated to increase the memory B cell pool without further affinity maturation [16]. In contrast, the response of IgM memory B cells after re-immunization was inhibited by high amounts of specific IgG in the serum masking the antigen [16]. In B cell receptor (BCR)-transgenic mice, the formation

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http://dx.doi.org/10.1016/j.ebiom.2016.09.003

Table 1
DENV-specific IgG in the plasma of patients detected by Western blot.

				Early		Acute		Convalescent	
Patient number	Patient ID	Infection	Current serotype of infection	Time	WB	Time	WB	Time	WB
1	10/63'	Secondary	DENV-2	45 h	–	4d	–	19d	–
2	10/50'	Secondary	DENV-3	17 h	–	6d	Env, prM	16d	Env, prM, NS1
3	E1392	Secondary	DENV-2	57 h	Env	3d	Env, prM	22d	Env, prM, NS1
4	E1183	Secondary	DENV-2	69 h	Env	4d	Env, prM	24d	Env, prM, NS1
5	E1407	Secondary	DENV-2	55 h	Env, prM	3d	Env, prM	16d	Env, prM, NS1
6	E1311	Secondary*	DENV-2	14 h	nd	7d	nd	15d	nd
7	C07	Secondary	DENV-2	–	–	6d	Env, prM, NS1	128d	Env, prM, NS1
8	E1291	Secondary	DENV-2	71 h	–	4d	Env, prM	23d	Env, prM, NS1
9	E1385	Primary	DENV-2	16 h	–	6d	nd	16d	Env, prM
10	C01	Primary	DENV-2	–	–	5d	nd	166d	Env, NS1
11	E1414	Primary	DENV-2	68 h	–	4d	Env, prM	17d	Env, prM, NS1
12	E1465	Primary	DENV-2	67 h	–	8d	nd	33d	nd

(–) Serum not available.
(nd) Not detected with 1:1000 dilution of plasma.
(') 10/63 and 10/50 have been described before in Ref. [8].
(*) This patient had no pre-existing DENV-binding IgG and was classified as secondary due to the highly EDIII-specific response of the plasmablasts.

of plasmablasts was facilitated by high affinity binding to the BCR [17] [18], a high antigen-to-B cell ratio, and a strong BCR signal [19,20], but this system is limited in that only one epitope can be studied. During a natural viral infection, B cells respond to multiple viral epitopes, and antibodies with both high and low neutralizing capacities can have similar affinities [21]. Thus, affinity alone does not determine the efficacy of an anti-viral response, and the different biological functions of plasmablasts versus memory B cells and long-lived plasma cells post primary infection are not clear.

In humans, plasmablasts appear in the blood five to seven days after infection or vaccination. Human plasmablasts have been studied extensively to monitor vaccine- or natural infection-induced specific B cell responses and to generate disease-specific human monoclonal antibodies [8,22–26]. Moreover, the plasmablast response was reported to be predictive of antibody titers at least during early convalescence [22,24]. Lavinder et al. studied whether plasmablasts or MBCs contributed to the serum antibody pool after tetanus vaccination and found little repertoire overlap, concluding that only a small fraction of plasmablasts and MBCs contributed to long-lived humoral immune memory [27].

The aim of the current study was to determine the repertoires and the potential protective capacity of plasmablasts versus memory B cells in the same individuals during acute dengue disease and after recovery, and to determine the developmental relationship between these two B cell subsets.

2. Methods

2.1. Patients

The study was approved by the Institutional Review Board of Singapore National Healthcare Group Ethical Domain (DSRB B/05/013), and patients gave written informed consent. Adult patients (age > 21 y) presenting at community primary care clinics with acute-onset fever (> 38.5 °C for 72 h) without rhinitis or clinically obvious alternative diagnoses were included in the study. Whole-blood samples were collected into EDTA-vacutainer tubes (Becton Dickinson) at recruitment (acute phase), at 3–7 d (defervescence), and at 3–4 wk. after fever onset (convalescence). Patients were diagnosed by DENV-specific RT-PCR. DENV-

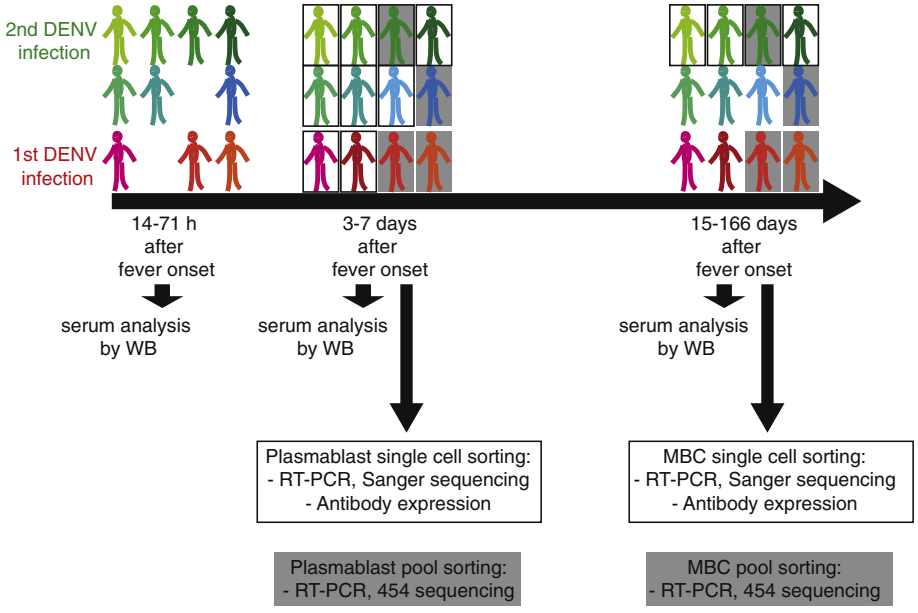

Fig. 1. Study setup and time points of sample collection. Dengue patients with a primary or secondary infection were enrolled into the study between 14 and 71 h after onset of fever. The same patients were recalled 4–7 days and 15–166 days after onset of fever. Each patient is color coded. Samples from patients with a black box were used for single B cell sorting, sequencing and Ab expression. Samples from patients with a grey-shaded box were used for pooled B cell sorting and 454 sequencing.

Please cite this article as: Appanna, R., et al., Plasmablasts During Acute Dengue Infection Represent a Small Subset of a Broader Virus-specific Memory B Cell Pool, EBioMedicine (2016), http://dx.doi.org/10.1016/j.ebiom.2016.09.003