WEEKLY EPIDEMIOLOGICAL REPORT

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Dengue-The Antibody Response (Part I)

This is the first in a series of two articles on antibody response to Dengue virus infection.

Background

Dengue viruses (DENV) are mosquito-borne flaviviruses and are the causative agents of Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF). Millions of people living in tropical and subtropical regions of the world are infected by DENV each year. Several hundred thousands of these infections progress to DHF, which is a life threatening disease. The DENV complex consists of 4 distinct but related viruses designated as serotypes.

DENVs display antibody epitopes (part of an antigen that is recognized by the immune system) that are unique to each serotype and epitopes that are shared between serotypes. People who have recovered from primary DENV infections develop robust antibody responses that cross react with the 4 serotypes. Despite the cross reactivity, antibodies only prevent re-infection by the same serotype (homologous serotype) and individuals are susceptible to a second infection with a different serotype (heterologous serotype). People experiencing a secondary dengue infection with a new serotype face a much greater risk of developing DHF indicating that pre-existing immunity to DENV can exacerbate disease.

Antibody Dependent Enhancement (ADE) of DENV is the most widely supported theory explaining the higher risk of DHF associated with secondary infection. Thus, the antibody response to DENV infection is complex, with potential to benefit or harm the host.

Dengue Virion Structure

The structural arrangement of viral surface proteins plays an important role in dictating how antibodies neutralize viruses. Dengue is an enveloped, positive-strand RNA virus that produces a spherical particle with a diameter of approximately 500 Angstroms (Angstrom=10^{-10} m or 0.1 nm). The viral envelope contains two integral membrane proteins, designated envelope (E) and pre membrane (prM). E protein binds to cellular receptors and mediates fusion of viral and cellular membranes during viral entry into cells. E protein is also the main target of neutralizing antibodies.

Individual subunits of E protein consist of three domains designated domains I (EDI), II (EDII) and III (EDIII). Domain III appears to be responsible for binding to cellular receptors as several mutations that affect receptor binding are located in this domain.

Each virus particle has 180 monomers of E that are organized into 90 tightly packed dimers that lie flat on the surface of the viral membrane. Individual E subunits are organized in 2, 3 and 5 fold axes of the structure of the virion. Thus, all the E protein subunits are not in identical environments on the viral surface and steric and other considerations will result in preferential interactions of some E subunits over others with receptors and antibodies.

Human Antibody Response Following Natural DENV Infection

People exposed to DENV infections have detectable specific antibody for decades if not longer. A large fraction of the response cross reacts with all 4 serotypes and even other flaviviruses. In fact the dominance of cross reactive antibodies precludes the use of simple antigen binding assays to identify a flavivirus responsible for infection. The functional, neutralizing antibody response is more specific and useful for identifying the flavivirus responsible for infection. Early studies on the durability of the immune response following DENV infection were conducted by Sabin in1952. Sabin infected naive volunteers with DENV2 and DENV 1and re-challenged these individuals with the homologous virus at dif-

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ferent times after the first infection. The subjects were protected up to 18 months (last time point of the study) from re-infection with the same serotype. However, when challenged with a heterologous serotype, cross protection only lasted for 2–3 months after the first infection. Heterologous infection not only produced clinical signs of the disease but also produced sufficient viremia for mosquitoes to acquire infection. Sabin's work demonstrated that protection was long term against the homologous serotype and only transient against a heterologous serotype. These observations are in agreement with natural history studies of dengue in endemic countries that indicate that primary infections are followed by a several month transient period of broad protection, and a long term protective response that is specific just to the infecting serotype. A recent study provides a molecular basis for this initial broad cross neutralizing response. These investigators demonstrated that early convalescent sera contain high concentrations of weakly neutralizing, cross reactive antibodies capable of forming large virus-antibody aggregates, which then binds to inhibitory FcγRIIB receptors on the surface of monocytes. The protection afforded by this class of antibody is likely to be transient because levels of cross reactive antibodies decline over time. In contrast, potently neutralizing, type-specific antibodies did not require the formation of aggregates for effective neutralization. Type specific neutralizing antibodies can be detected even 60 years after a primary infection.

Several months after a primary dengue infection, individuals are susceptible to a secondary infection with a new serotype. A hallmark of secondary dengue is a more rapid and elevated antibody response compared to the primary response. The rapid and elevated response is caused by the stimulation of memory B-cells from the primary infection. The first antibodies that appear following a secondary infection neutralize the serotype responsible for the primary infection better than the second virus. This phenomenon has been termed "original antigenic sin", although the molecular basis and mechanisms responsible are incompletely understood. Over time, the neutralizing antibody response broadens and a key feature of secondary dengue is a long-lasting response that neutralizes multiple serotypes including serotypes that have not previously infected the individual. Tertiary DENV infections have been documented only rarely, further supporting the notion that secondary infections stimulate long term cross neutralizing antibody that may even be effective against serotypes not encountered previously.

Investigators have also characterized the kinetics and isotypes of the DENV-specific serum antibody response in infected people. Following a primary DENV infection, DENV-specific IgM antibodies appear 4–5 days after the onset of fever and are measurable for up to 3 months. IgG antibodies first appear about a week after the onset of fever. The IgG response peaks several weeks after infection and then declines to lower levels that persist for decades if not longer. DENV infection mainly induces IgG1 and IgG3 subclasses of antibodies.

The serum antibody responses are different following primary and secondary DENV infections. Antibodies produced during a second infection arise from naive B-cells and memory B-cells generated from the primary infection. In secondary infections, the stimulation of B-cell memory leads to a rapid rise in DENV-specific IgG that is measurable even on the first day of symptoms. Moreover, DENV-specific serum IgG titers are much higher in secondary compared to primary infections. For reasons that are not completely understood, in secondary dengue the IgM response is variable, and some cases undetectable.

Intra-Serotype Strain Variation and DENV Neutralization

DENVs within each serotype are genetically diverse and classified into distinct genotypes with different geographical distributions and pathogenic potential. Despite this genetic variability, it is widely assumed that neutralizing antibody epitopes are conserved among strains belonging to the same serotype. In fact, the current strategy for developing dengue vaccines is based on the assumption that a neutralizing immune response directed to a single strain will protect against most if not all strains of DENV within the serotype. The idea that strain variation within a serotype does not affect neutralization is mainly based on the observation that in human cohort studies one rarely, if ever, observes re-infection with the same serotype. It is argued that this observation alone does not mean intra-serotype strain variation is irrelevant for neutralization because most cohort studies have been done in areas where each serotype is represented by the circulating single genotype. It is necessary to conduct prospective studies that specifically assess what happens when a new genotype is introduced into a population with pre-existing immunity to the serotype. Several recent laboratory based studied indicate that intra-serotype variation can lead to large differences in antibody neutralization. A study was carried out on monkeys using a panel of viruses representing the 4 serotypes and genotypes within each serotype, and it showed large differences in neutralization titer when comparing different genotypes of DENV3. In a study of pediatric dengue cases in Thailand, investigators observed significant differences in the ability of sera to neutralize reference and clinical strains of DENV3. Thus, the current paradigm that neutralizing antibody epitopes are conserved within each serotype needs to be tested more vigorously both in the field and in the laboratory.

In Vitro versus in Vivo Neutralization

Studies are needed to assess the relationship between cell culture antibody neutralization of DENVs and in vivo protection from infection and disease. Typically cell culture neutralization is based on antibodies binding to the virion and directly interfering with infection. In vivo, the situation is more complex and antibodies can interact with other components of the immune system such as complement and Fc receptors (found in B lymphocytes, natural killer cells, macrophages, neutrophils and mast cells), which can augment or suppress virus neutralization. Moreover, antibodies can also harness cellular mechanisms such as phagocytosis and Antibody Dependent Cellular Cytotoxicity (ADCC) to control DENV infection. Despite these differences, some studies have reported on a strong correlation between in vitro neutralization potency and in vivo protection both with monoclonal antibodies and polyclonal sera. A recent study of infants with maternally derived DENV- specific antibody indicated that an in-vitro neutralization titer of 1:50 is predictive of protection in vivo as well. However, some flavivirus antibodies with poor naturalizing activity in cell culture can protect from disease in animal models. Further studies are needed to define the main mechanisms by which antibodies protect people from severe dengue disease.

Source

The Human Antibody Response to Dengue Virus Infection, available from *http://www.mdpi.com/1999-4915/3/12/2374/pdf*

Compiled by Dr. Madhava Gunasekera of the Epidemiology Unit

to be continued

Table 1: Vaccine-preventable Diseases & AFP

29th September – 05th October 2012 (40thWeek)

Disease	No. of Cases by Province									Number of cases during current week in 2012	Number of cases during same week in 2011	Total number of cases to date in 2012	Total number of cases to date in 2011	Difference between the number of cases to date in 2012 & 2011
	W	C	S	N	E	NW	NC	U	Sab					
Acute Flaccid Paralysis	00	00	01	00	00	00	00	00	00	01	02	61	72	- 15.2 %
Diphtheria	00	00	00	00	00	00	00	00	00	-	-	-	-	-
Measles	01	00	00	00	00	01	00	00	00	02	01	49	110	- 55.4 %
Tetanus	01	00	00	00	00	00	00	00	00	01	01	10	21	- 52.4 %
Whooping Cough	01	00	01	00	00	00	00	00	02	04	02	86	45	+ 91.1 %
Tuberculosis	125	36	22	26	13	02	05	10	34	273	152	6687	7203	- 07.2 %

Table 2: Newly Introduced Notifiable Disease

29th September – 05th October 2012 (40th Week)

Disease	No. of Cases by Province									Number of cases during current week in 2012	Number of cases during same week in 2011	Total number of cases to date in 2012	Total number of cases to date in 2011	Difference between the number of cases to date in 2012 & 2011
	W	C	S	N	E	NW	NC	U	Sab					
Chickenpox	31	02	14	03	01	06	04	02	04	67	52	3540	3417	+ 03.6 %
Meningitis	06 CB=2 KL=4	01 ML=1	03 HB=1 GL=2	01 JF=1	00	00	01 AP=1	00	01 KG=1	13	10	644	695	- 07.3 %
Mumps	09	02	08	03	05	10	05	02	04	48	37	3781	2501	+ 51.2 %
Leishmaniasis	00	00	17 HB=13 MT=4	02 MN=1 VU=1	00	01 KN=1	11 AP=8 PO=3	00	00	30	31	844	641	- 31.7 %

Key to Table 1 & 2

Provinces: **W**: Western, **C**: Central, **S**: Southern, **N**: North, **E**: East, **NC**: North Central, **NW**: North Western, **U**: Uva, **Sab**: Sabaragamuwa.

DPDHS Divisions: **CB**: Colombo, **GM**: Gampaha, **KL**: Kalutara, **KD**: Kandy, **ML**: Matale, **NE**: Nuwara Eliya, **GL**: Galle, **HB**: Hambantota, **MT**: Matara, **JF**: Jaffna, **KN**: Killinochchi, **MN**: Mannar, **VA**: Vavuniya, **MU**: Mullaitivu, **BT**: Batticaloa, **AM**: Ampara, **TR**: Trincomalee, **KM**: Kalmunai, **KR**: Kurunegala, **PU**: Puttalam, **AP**: Anuradhapura, **PO**: Polonnaruwa, **BD**: Badulla, **MO**: Moneragala, **RP**: Ratnapura, **KG**: Kegalle.

Data Sources:

Weekly Return of Communicable Diseases: Diphtheria, Measles, Tetanus, Whooping Cough, Chickenpox, Meningitis, Mumps.

Special Surveillance: Acute Flaccid Paralysis.

Leishmaniasis is notifiable only after the General Circular No: 02/102/2008 issued on 23 September 2008.

Dengue Prevention and Control Health Messages

Check the roof gutters regularly for water collection where dengue mosquitoes could breed.

Table 4: Selected notifiable diseases reported by Medical Officers of Health

29th September – 05th October 2012 (40th Week)

DPDHS Division	Dengue Fever / DHF*		Dysentery		Encephalitis		Enteric Fever		Food Poisoning		Leptospirosis		Typhus Fever		Viral Hepatitis		Human Rabies		Returns Received
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	%
Colombo	144	7847	4	122	0	8	5	183	0	43	6	163	1	5	1	100	0	5	**85**
Gampaha	121	6316	1	73	0	13	2	55	3	41	6	203	1	21	6	269	0	0	**100**
Kalutara	32	2228	0	85	0	4	1	42	0	28	9	211	1	4	0	30	0	2	**69**
Kandy	39	1990	2	103	0	2	1	21	0	56	2	61	1	102	6	94	0	0	**87**
Matale	7	433	1	76	0	5	0	9	0	7	0	33	0	3	0	32	0	0	**67**
Nuwara	2	283	2	163	0	3	0	25	0	8	0	31	0	59	0	18	0	1	**85**
Galle	10	1252	3	109	0	6	1	13	0	17	2	104	0	61	1	3	0	0	**89**
Hambantota	14	468	1	38	0	2	0	06	0	30	0	64	1	48	0	19	0	0	**83**
Matara	38	1400	0	67	0	8	0	19	2	23	10	152	0	68	6	120	0	0	**100**
Jaffna	7	369	5	163	0	14	0	309	0	81	0	2	0	251	0	16	0	1	**100**
Kilinochchi	0	72	0	15	0	2	0	28	0	43	0	4	0	29	0	4	0	1	**75**
Mannar	0	126	0	64	0	4	0	24	0	16	0	22	0	42	0	2	0	0	**60**
Vavuniya	5	76	0	27	0	21	2	11	0	17	0	18	0	3	0	1	0	0	**50**
Mullaitivu	0	21	0	16	0	1	0	9	0	3	0	3	0	5	0	1	0	0	**40**
Batticaloa	2	610	7	210	0	2	0	15	0	307	0	8	0	0	1	8	0	4	**86**
Ampara	2	125	0	75	0	2	0	6	2	12	0	27	0	0	0	3	0	0	**57**
Trincomalee	0	129	3	166	0	2	0	16	0	13	0	37	0	17	0	4	0	0	**100**
Kurunegala	38	2078	4	165	0	15	3	84	0	35	1	124	0	27	1	122	0	4	**81**
Puttalam	15	1109	1	80	0	7	0	12	0	10	2	38	0	15	0	5	0	2	**50**
Anuradhapu	2	307	2	71	0	7	0	13	2	20	0	77	0	23	1	56	0	1	**53**
Polonnaruw	4	206	2	57	0	2	1	4	0	121	1	43	0	3	1	38	0	1	**71**
Badulla	14	299	1	103	0	4	0	49	0	3	0	36	5	103	1	41	0	0	**76**
Monaragala	3	224	1	55	0	5	1	22	0	7	0	60	2	73	2	166	0	2	**91**
Ratnapura	42	3339	4	193	0	25	1	45	0	12	5	257	1	38	5	104	0	2	**89**
Kegalle	39	2248	0	54	0	9	0	21	0	10	5	149	2	56	6	485	0	0	**91**
Kalmune	1	174	3	226	0	1	0	6	0	85	0	8	0	1	0	10	0	3	**54**
SRI LANKA	**581**	**33729**	**47**	**2576**	**00**	**174**	**18**	**1047**	**09**	**1048**	**49**	**1935**	**15**	**1057**	**38**	**1751**	**00**	**29**	**80**

Source: Weekly Returns of Communicable Diseases WRCD).
***Dengue Fever / DHF** refers to Dengue Fever / Dengue Haemorrhagic Fever.
****Timely** refers to returns received on or before 05th October , 2012 Total number of reporting units 329. Number of reporting units data provided for the current week: 266
A = Cases reported during the current week. **B** = Cumulative cases for the year.

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Comments and contributions for publication in the WER Sri Lanka are welcome. However, the editor reserves the right to accept or reject items for publication. All correspondence should be mailed to The Editor, WER Sri Lanka, Epidemiological Unit, P.O. Box 1567, Colombo or sent by E-mail to **chepid@sltnet.lk.**

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