WEEKLY EPIDEMIOLOGICAL REPORT

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Dengue -The Antibody Response (Part II)

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

B-Cell Subsets Involved in the Humoral Response to DENV

As discussed earlier, most work to-date on the human antibody response to DENV has focused on circulating serum antibody and Monoclonal Antibodies generated from the memory B-cell pool. Studies are needed to identify the actual B-cell subsets activated by dengue and to characterize the functional importance of antibody produced from different B-cell populations. Most antibodies stimulated by viruses are classical T-dependent responses derived from follicular B-cells. The main antibody response to DENV is also likely to involve T-dependent follicular (B-2) B-cells, which differentiate into long lived plasma cells and memory B-cells. Recent studies indicate that less well studied B-cell subsets such as marginal zone B-cells and B1a and B1b cells, which give rise to natural and T-independent antibody responses provide protection from viruses. Studies are needed to assess if similar responses constitute important components of the response to DENV as well. In this regard, the recent observation that many human flavivirus antibodies recognize epitopes preserved on the intact virion but not recombinant E protein is intriguing as such antibodies may be produced by the multivalent virus particles directly activating B-cells without any T-cell help. DENV infection also inhibits type I interferon response and suppresses antigen presentation by myeloid cells and these effects are likely to influence the quality of the adaptive immune response, including antibody production. It is necessary to invest in human studies and animal models to characterize B-cell subsets involved in the response to DENV, with particular attention to how these responses differ in primary versus secondary cases, or severe versus mild disease cases.

Role of Antibodies in Enhancing DENV Infection and Disease

Many studies in different regions of the world have documented that individuals exposed to secondary infections are at greater risk of developing severe disease compared to individuals exposed to primary infections. The leading theory proposed to explain the increased risk of severe disease in secondary cases is Antibody Dependent Enhancement (ADE), which postulates that weakly neutralizing antibodies from the first infection bind to the second serotype and enhance infection of FcγR bearing myeloid cells such as monocytes and macrophages. The fact that secondary infections lead to a higher serum viremia and a greater risk of severe disease compared to primary infections strongly suggests that pre-existing immunity (not necessarily antibody) to DENV is a risk factor for severe dengue. The most compelling evidence for ADE has come from studies with infants, who have passively acquired antibodies to DENV from their mothers. Soon after birth, high levels of maternal antibodies appear to protect infants from dengue. Infants born to dengue immune mothers are at greatest risk of developing severe dengue between 6–12 months after birth and this has been attributed to maternal antibody decaying to low, sub -neutralizing levels that enhance DENV. Studies with older children experiencing secondary infections also provide evidence in support of ADE and severe disease. In one study, the ability of immune sera collected just before a second infection to enhance DENV in cell culture was positively correlated with the risk of severe disease. However, not all human studies support the ADE theory of DHF/DSS. While it is difficult to directly compare different studies because of differences in study design and methods, the ADE hypothesis is biologically plausible and supported by sufficient evidence to justify conducting more human cohort studies specifically designed to test the ADE hypothesis.

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It is challenging to test the ADE hypothesis in animal models because DENV replicates poorly in animals, and DHF and DSS are difficult to reproduce in animal models. Nonetheless, a few studies indicate that antibody enhanced infection and severe disease can be reproduced in some animals. Studies with non-human primates have demonstrated that animals treated with sub-neutralizing levels of antibody develop higher serum viremia compared to untreated animals.

However, attempts to reproduce DHF in non-human primates have been unsuccessful. Investigators have attempted with some success to develop mouse models of DENV infection and disease. The most successful mouse model of antibody enhanced severe disease is based on infecting interferon receptor-deficient AG129 mice with a mouse adapted strain of DENV2. AG129 mice treated with anti-dengue monoclonal antibodies or polyclonal sera and then infected with DENV developed a lethal vascular leak disease, with similarities to DHF. The cells involved with ADE driven infection were Fc receptor bearing cells including sinusoidal endothelial cells in the liver. In addition to vascular leakage, the disease was characterized by elevated level of cytokines (TNFα, IL-6, and IL-10) and thrombocytopenia, which is similar to severe DENV illness in humans. While specific cellular mechanisms leading to vascular leakage are likely to be different in humans and mice, especially mice deficient in interferon receptors, these animal studies establish that antibodies can enhance viral replication and induce host cytokine responses that lead to clinical outcomes similar to DHF/DSS. Further, development of appropriate animal models, including mice reconstituted with human immune cells, is an ongoing and exciting area of dengue research.

The ADE phenomenon can be reproduced in cell culture models. Fcγ receptor bearing human cell lines such as monocytes and macrophages, which are not efficiently infected with DENV alone, become highly permissive to infection in the presence of sub-neutralizing antibody concentrations. ADE has been well demonstrated with monoclonal antibodies and polyclonal sera in vitro using Fcγ receptor bearing human cells such as K562, U937, primary human monocytes, macrophages and dendritic cells. Initially it was believed that ADE simply resulted from a greater number of infected cells producing more infectious virions (extrinsic ADE). However, recent studies with THP-1 cells (a human acute monocytic leukemia cell line) indicate that the phenomenon is more complex. DENVs entering THP-1 cells via Fc receptors suppressed type I interferon responses and the activation of cellular antiviral molecules more effectively than DENV infecting the same cells in the absence of antibody. Moreover, THP1 cells infected in the presence of antibody produced more infectious virus per infected cell compared to cells infected in the absence of antibody. This phenomenon, which has been termed "intrinsic ADE" demonstrates that antibody mediated infection leads to a suppression of the antiviral state within the infected cell and the release of a greater quantity of infectious virions by each infected cell compared to cells infected by antibody-independent entry. With THP-1 cells, antibody dependent infections inhibited type I interferon responses and increased levels of the suppressive cytokine IL-10. In studies with primary human Peripheral Blood Mononuclear Cells (PBMCs), one group did not observe any differences in type Type I Interferon or IL-10 levels whereas another group observed decreased levels of type I interferon and increased levels of IL-6. The picture emerging from these studies is that antibody-complexed dengue viruses infecting cells via Fcγ receptors leads to the suppression of cellular antiviral responses, but further studies are needed to better define the cellular pathways and mechanisms that contribute to this phenotype.

Properties of DENV Enhancing Antibodies

It is well established that almost any dengue specific Monoclonal Antibodies used at sub-neutralizing concentrations can enhance infection of cultured cells expressing appropriate Fcγ receptors. Studies are needed to identify specific antibody sub-populations in dengue immune human sera that drive ADE in cell culture and animal models and, potentially, in people exposed to secondary infections. It is important to keep in mind that during a secondary DENV infection, ADE is likely to occur at circulating antibody concentrations present during the acute phase of infection. A recent study demonstrated a quantitative difference in the ability of primary immune sera to enhance a homologous versus heterologous DENV serotype. Sera had to be diluted to artificially low concentrations to enhance the homologous virus whereas a heterologous serotype was enhanced at high serum concentrations, likely to be encountered by a virus responsible for a secondary infection. The actual target(s) of antibodies in human immune sera that enhance DENV infection have not been identified.

In this regard, predominance of prM Human Monoclonal Antibodies has raised the intriguing possibility that these antibodies play a pivotal role in enhancing human DENV infections. DENV produced in cell culture is a complex mix of immature, partially mature and fully mature virions and these preparations are efficiently enhanced in vitro and in mice by prM antibodies. By identifying the main antibodies in immune sera with potential for enhancing dengue at physiological concentrations, it will be possible to design vaccines that neutralize without potential for enhancement.

Summary

People who have recovered from primary DENV infections develop antibody responses that cross react with the 4 serotypes. Despite the cross reactivity, antibodies only prevent re-infection by the same serotype (homologous 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.

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

Table 1: Vaccine-preventable Diseases & AFP

06th – 12th October 2012 (41stWeek)

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	01	01	00	00	00	01	01	00	04	02	64	74	- 13.5 %
Diphtheria	00	00	00	00	00	00	00	00	00	-	-	-	-	-
Measles	00	00	00	00	00	01	00	00	00	01	01	50	111	- 54.9 %
Tetanus	01	00	00	00	00	00	00	00	00	00	01	11	22	- 50.0 %
Whooping Cough	01	00	01	00	00	00	00	00	02	00	01	86	45	+ 113.3 %
Tuberculosis	08	28	15	04	14	27	19	10	11	136	72	6823	7275	- 06.2 %

Table 2: Newly Introduced Notifiable Disease

06th – 12th October 2012 (41stWeek)

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	11	05	09	02	07	12	04	05	05	60	55	3611	3494	+ 03.3 %
Meningitis	03 CB=1 KL=2	00	00	01 JF=1	01 AM=1	00	02 AP=1 PO=1	01 BD=1	01 RP=1	09	13	657	709	- 07.3 %
Mumps	10	00	03	02	03	02	10	08	03	41	46	3823	2563	+ 49.2 %
Leishmaniasis	00	00	03 HB=1 MT=2	00	01 TR=1	01 KN=1	10 AP=8 PO=2	00	00	15	06	864	653	- 32.1 %

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

You have a duty and a responsibility in preventing dengue fever. Make sure that your environment is free from water collections where the dengue mosquito could breed.

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

06th – 12th October 2012 (41stWeek)

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	105	7981	3	125	0	8	3	186	0	43	1	165	0	5	1	102	0	5	**85**
Gampaha	69	6400	0	73	0	14	0	55	0	41	9	214	0	21	1	270	0	0	**60**
Kalutara	45	2277	4	89	0	4	0	42	0	28	7	218	0	4	0	30	0	2	**85**
Kandy	39	2048	3	106	0	2	1	22	0	56	0	61	2	105	2	96	0	0	**96**
Matale	8	444	0	76	0	5	2	11	1	8	3	36	0	3	0	32	0	0	**100**
Nuwara	6	289	2	165	0	3	0	25	0	8	0	31	1	60	0	18	0	1	**69**
Galle	18	1274	3	112	0	6	0	13	0	17	2	106	3	64	0	3	0	0	**89**
Hambantota	8	476	0	38	0	2	0	6	0	30	1	65	1	49	1	20	0	0	**100**
Matara	55	1465	4	71	0	8	0	19	0	23	6	158	2	70	4	124	0	0	**100**
Jaffna	49	418	4	167	0	14	2	313	0	82	0	2	1	253	0	16	0	1	**83**
Kilinochchi	0	75	1	19	0	2	0	29	0	43	0	4	1	30	0	4	0	1	**50**
Mannar	0	126	0	66	0	4	0	35	0	16	0	23	0	42	0	2	0	0	**40**
Vavuniya	0	76	2	30	0	21	0	12	2	19	0	18	0	3	0	1	0	0	**75**
Mullaitivu	0	22	0	16	0	1	0	11	0	3	0	3	0	5	0	1	0	0	**40**
Batticaloa	5	616	12	223	0	2	0	15	0	307	0	8	0	0	0	8	0	4	**79**
Ampara	0	126	0	75	0	2	0	6	0	12	0	27	0	0	0	3	0	0	**29**
Trincomalee	2	131	6	172	0	2	0	16	0	13	0	37	0	17	0	4	0	0	**92**
Kurunegala	106	2218	3	168	1	16	1	85	1	36	2	127	0	28	2	124	0	4	**88**
Puttalam	34	1157	3	84	1	8	0	12	0	10	0	38	0	15	0	5	0	2	**58**
Anuradhapu	7	318	0	71	0	7	0	13	0	20	0	77	0	23	1	57	0	1	**68**
Polonnaruw	3	210	2	60	0	2	0	4	0	121	2	45	0	3	1	40	0	1	**71**
Badulla	6	308	1	105	0	4	0	49	0	3	0	36	1	104	1	42	0	0	**88**
Monaragala	4	229	3	58	1	6	0	23	0	7	0	60	2	75	2	168	0	2	**82**
Ratnapura	22	3364	5	198	0	25	1	46	0	12	3	260	0	38	2	106	0	2	**67**
Kegalle	22	2272	0	54	0	9	2	23	0	10	1	150	0	56	11	496	0	0	**82**
Kalmune	0	175	4	237	0	1	0	6	0	86	0	9	0	1	0	10	0	3	**85**
SRI LANKA	**613**	**34495**	**65**	**2658**	**03**	**178**	**12**	**1077**	**04**	**1054**	**37**	**1978**	**14**	**1074**	**29**	**1782**	**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 12th October , 2012 Total number of reporting units 329. Number of reporting units data provided for the current week: 267
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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