Published in final edited form as:

Viral Immunol. 2007 December ; 20(4): 672–675. doi:10.1089/vim.2007.0050.

Antibody to Dengue 1 Detected More Than 60 Years after Infection

Allison Imrie¹, Janet Meeks², Alexandra Gurary², Munkhzul Suhkbaatar¹, Thang Thua Truong³, C. Bruce Cropp³, and Paul Effler⁴

¹Department of Public Health Sciences, John A. Burns School of Medicine, University of Hawaii at Manoa

²Department of Pediatrics, John A. Burns School of Medicine, University of Hawaii at Manoa

³Department of Tropical Medicine, Medical Microbiology, and Pharmacology, John A. Burns School of Medicine, University of Hawaii at Manoa

⁴Disease Outbreak Division, Hawaii State Department of Health, Honolulu, Hawaii

Abstract

We investigated the duration of humoral responses to dengue virus infection in individuals who recalled experiencing dengue fever-like illnesses at the time of the Second World War, when dengue fever epidemics occurred throughout the Pacific and Southeast Asia. In July 1943 dengue fever reappeared in Hawaii following an interval of 31 years. Over the next 12 months a total of 1498 locally transmitted cases were reported, and at least 46 imported cases were identified, most of which were among members of the military returning from the Pacific Theatre of the war. Serum samples collected in 2005, more than 60 years after onset of symptoms, were tested for the presence of dengue-specific antibodies using a rapid ELISA test, and by plaque reduction neutralization test. Four of seven samples were positive for dengue-specific IgG and demonstrated neutralization titers ≥ 160 to dengue 1. We describe the existence of dengue-specific antibodies in the serum of people infected more than 60 years earlier.

BRIEF REPORT

Dengue Viruses Are mosquito-borne single-stranded RNA viruses that belong to the family Flaviviridae. Infection with any of the four dengue serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) can cause a spectrum of illnesses ranging from asymptomatic infection to severe hemorrhagic disease and shock syndrome, which can be fatal. Immunity to one dengue serotype following a first, or primary, infection does not protect against subsequent infection with any of the other three serotypes, and indeed, epidemiological observations suggest that severe disease occurs more frequently in the setting of secondary infection with heterologous virus (reviewed in 6,7). The pathogenesis of the severe forms of disease is not fully understood, but one hypothesis is based on the idea of antibody-dependent enhancement, in which preexisting non-neutralizing antibodies induced during the first dengue infection enhance infection of mononuclear cells during the second infection via cell surface FcγR (9, reviewed in 13). Thus, persistence of dengue-specific antibody may be a significant risk factor for development of severe disease in countries where dengue is hyperendemic.

© Mary Ann Liebert, Inc.

Address reprint requests to: Dr. Allison Imrie, University of Hawaii, Public Health Sciences, Honolulu, HI 96813, imrie@pbrc.hawaii.edu.

We investigated the duration of humoral responses to dengue virus infection in individuals who recalled experiencing dengue fever-like illnesses (DFLIs) at the time of the Second World War, when dengue epidemics were occurring throughout the Pacific and Southeast Asia (4, reviewed in 6). In 1943 dengue fever reappeared in Hawaii following an interval of 31 years since the last epidemic in 1912. The first case, in a resident of Waikiki, Honolulu, was reported to the Board of Health of the Territory of Hawaii on August 5 (4·5). The disease was thought to have been introduced by airline pilots staying in a Waikiki hotel who had arrived in Honolulu on July 19 from Suva, Fiji, where an epidemic of dengue fever was occurring (4·5·14). One pilot was symptomatic with DFLI upon arrival and the other became ill shortly thereafter. Over the next 12 months a total of 1498 locally transmitted cases were reported, all but two of which occurred on the island of Oahu (22). At least 46 imported cases were identified, most of which (37/46, 80%) were among members of the military returning from the Pacific Theatre of the war.

Seven people, four men and three women, responded to a newspaper article seeking contact with persons living in Hawaii who recalled being ill during the Second World War (11). Six reported experiencing severe, often debilitating flu-like symptoms and maintained vivid memories of their illness. The seventh individual was a child at the time of the epidemic and was subsequently told by her mother, who experienced a DFLI, that she had been ill at that time. Prior to enrolment in this study, approved by the University of Hawaii at Manoa institutional review board, all subjects gave written informed consent.

Serum samples collected in 2005, more than 60 years after onset of symptoms, were tested for the presence of dengue-specific antibodies using a rapid ELISA test (Panbio, Sinnamon Park, Queensland, Australia), and by 90% plaque reduction neutralization test in a 6-well plate format utilizing Vero cells (19). Samples were tested in duplicate in twofold dilutions starting at 1:10, for a final input dose of 100 pfu of each of the four dengue virus serotypes. Back titrations of each virus were included to confirm 90% plaque reduction. Four of seven samples were positive for dengue-specific IgG and demonstrated neutralization titers of ≥ 160 against dengue 1 (Table 1).

Three men were serving in the U.S. military, in the Western Pacific region. Subject 1 recalled that a large number of his fellow soldiers were ill at the same time he was, and that the medical officer accompanying their evacuation from Guam back to Hawaii told him they had dengue fever. Subject 2 was based in northern Australia and fought in New Guinea. The PRNT₉₀ response to DENV-2 measured for this individual may reflect infection with both viruses, since both were present in the region at the time (6), or alternatively, may reflect cross-reactivity in the neutralization assay (18). Subject 4 convalesced in a military hospital in Queensland, Australia, where he recalled receiving a diagnosis of dengue. The three women were all in Hawaii at the time they became ill and have lived there since; one, Subject 3, never traveled outside Hawaii. The high DENV-4 titers measured for this individual may be a result of unrecognized transmission of dengue in Hawaii between 1944 and 2001, but in light of the high DENV-1 titer, more likely reflects heterotypic cross-reactive antibody.

One individual (Subject 1) has visited Asia and Africa in the years since 1944, for brief periods, and it is possible that he was infected during these visits, but it is more likely, based on the epidemiology of dengue during the years of the Second World War, and his recollected experiences, that his neutralization data reflect infection in 1944.

Neutralizing antibody levels were not detected in two of seven subjects, indicating either a lack of prior infection, or a decline in titers to below detectable levels. Sera from the remaining individual, Subject 4, neutralized DENV-1 and DENV-2 with titers of 1:10 and

1:20, respectively, and IgG ELISA was negative. It is possible that this man was infected with DENV-1 and/or DENV-2 while he was in the Western Pacific region during World War II, and that his antibody titers had declined more rapidly compared with the other subjects in this study.

Several lines of evidence corroborate our claim that the neutralization data reflect dengue infections patients recall from 1943–1944 and not a more recent infection during Hawaii's outbreak of 2001–2002 (2·12). First, during the 2001–2002 outbreak 1644 persons with dengue fever–like symptoms were tested to find just 122 infections; given the extensive case finding effort it is unlikely that widespread dengue transmission occurred and went undetected. Second, all subjects in the present study live on Oahu; during the 2001–2002 outbreak only 26 (4%) of more than 700 persons tested from Oahu had dengue. Most of these infections (77%) were from family clusters in two adjacent communities on the windward side of the island—an area where none of our subjects resided. Third, all of the persons with dengue infections in the 2001–2002 outbreak reported symptoms and nearly all (89%) met the clinical criteria for dengue fever; none of the subjects in the present study reported experiencing a clinically compatible illness during the 2001–2002 outbreak. Fourth, a study of more than 3000 visitors to Hawaii during the peak of the 2001–2002 outbreak found zero infections per 358 person-days of exposure (upper 95% confidence limit: 3.0 cases per person-year) (20). Taken in aggregate, these data strongly suggest that widespread undetected dengue transmission did not occur in Hawaii during 2001–2002; therefore the neutralization data we report likely represent infections that occurred more than 60 years prior.

Antibody responses following acute viral infection in humans have been shown to persist for many years. Because of the high frequency of reinfection by many viruses it can be difficult to accurately determine the duration of the humoral response; however, recent studies of smallpox immunity have demonstrated clearly that antibodies induced by immunization with vaccinia virus can be maintained for up to 75 years (10). Memory B cells, which likely replenish long-lived antibody-secreting plasma cells, have been detected more than 50 years following smallpox vaccination (1). Similarly, long-term persistence of anti-flavivirus antibodies has been demonstrated in several studies. In a study of World War II veterans, Poland *et al.* detected neutralizing antibody 30–35 years after immunization with 17D yellow fever virus vaccine (17). Serum samples obtained up to 48 years following experimental infection with dengue of human volunteers in Osaka (15), and of American Army recruits in the Philippines (8), showed persistence of neutralizing antibody for up to 48 years, as measured by plaque reduction, complement fixation, and hemagglutination inhibition assays. Follow-up studies of individuals thought to have been infected during dengue epidemics in 1927–1928 in Greece (16) and in 1942–1944 in Japan (3·21) showed that neutralizing antibodies may persist for up to 46 years following natural infection. We describe the existence of dengue-specific antibodies in the serum of people naturally infected with dengue more than 60 years earlier, extending the findings of these earlier studies.

Acknowledgments

This publication was made possible by grant P20RR018727 from the National Center for Research Resources of the National Institutes of Health.

REFERENCES

1. Crotty S, Felgner P, Davies H, Glidewell J, Villarreal L, Ahmed R. Cutting edge: Long-term B cell memory in humans after smallpox vaccination. *J Immunol.* 2003; 171:4969–4973. [PubMed: 14607890]

2. Effler PV, Pang L, Kitsutani P, et al. and the Hawaii Dengue Outbreak Investigation Team. Dengue fever, Hawaii, 2001–2002. *Emerg Infect Dis.* 2005; 11:742–749. [PubMed: 15890132]
3. Fujita N, Yoshida K. Follow-up studies on dengue endemic in Nagasaki, Japan: detection of specific antibodies in the serum taken more than 30 years after a single attack of dengue. *Kobe J Med Sci.* 1979; 25:217–224. [PubMed: 522428]
4. Gilbertson WE. Dengue fever epidemic. *Hawaii Health Messenger.* 1944; 4:2.
5. Gilbertson WE. Sanitary aspects of the control of the 1943–1944 epidemic of dengue fever in Honolulu. *Am J Pub Health Nat Health.* 1945; 35:261–270.
6. Gubler DJ. Dengue/dengue hemorrhagic fever: history and current status. *Novartis Found Symp.* 2006; 277:3–16. discussion 16–22, 71–73, 251–253. Review. [PubMed: 17319151]
7. Guzman GG, Kouri K. Dengue: an update. *Lancet Infect Dis.* 2002; 2:33–42. Review. [PubMed: 11892494]
8. Halstead SB. Etiologies of the experimental dengues of Stiler and Simmons. *Am J Trop Med Hyg.* 1974; 23:974–982. [PubMed: 4615598]
9. Halstead SB. Pathogenesis of dengue: challenges to molecular biology. *Science.* 1988; 239:476–481. [PubMed: 3277268]
10. Hammarlund E, Lewis MW, Hansen SG, et al. Duration of antiviral immunity after smallpox vaccination. *Nat Med.* 2003; 9:1131–1137. [PubMed: 12925846]
11. Honolulu Star Bulletin. 2005 <http://starbulletin.com/2005/03/16/news/story12.html>.
12. Imrie A, Zhao Z, Bennett SN, Kitsutani P, Laille M, Effler PV. Molecular epidemiology of dengue in the Pacific: Introduction of two distinct strains of dengue 1 into Hawaii. *Ann Trop Med Parasit.* 2006; 100:327–336. [PubMed: 16762113]
13. Innis, BL. Antibody responses to virus infection. In: Gubler, DJ.; Kuno, G., editors. *Dengue and Dengue Hemorrhagic Fever.* New York: CAB International; 1997. p. 221–243.
14. Nance FD. Dengue fever: II. *Hawaii Med J.* 1943; 6:295.
15. Okuno Y, Fukunaga T, Tadano M, Fukai K, Ikeda T, Sekii K, Arivoshi H. Serological studies on volunteers inoculated experimentally with a dengue virus strain in 1943. *Biken J.* 1983; 26:161–163. [PubMed: 6678151]
16. Papaevangelou G, Halstead SB. Infections with two dengue viruses in Greece in the 20th century. Did dengue hemorrhagic fever occur in the 1928 epidemic? *Am J Trop Med Hyg.* 1977; 80:46–51.
17. Poland JD, Calisher CH, Monath TP, Downs WG, Murphy K. Persistence of neutralizing antibody 30–35 years after immunization with 17D yellow fever vaccine. *Bull World Health Organ.* 1981; 59:895–900. [PubMed: 6978196]
18. Rosen L. Dengue in Greece in 1927 and 1928 and the pathogenesis of dengue hemorrhagic fever: new data and different conclusions. *Am J Trop Med Hyg.* 1986; 35:642–653. [PubMed: 3518510]
19. Russell PK, Nisalak A, Sukhavachana P, Vivona S. Dengue virus neutralization by the plaque reduction neutralization test. *J Immunol.* 1967; 99:291–296. [PubMed: 4961907]
20. Smith CE, Tom T, Sasaki J, Ayers T, Effler PV. Dengue risk among visitors to Hawaii during an outbreak. *Emerg Infect Dis.* 2005; 11:750–756. [PubMed: 15890133]
21. Tadano M, Okuno Y, Fukunaga T, Fukai K. Retrospective serological studies on dengue epidemics in Osaka and Okinawa. *Biken J.* 1983; 26:165–167. [PubMed: 6378175]
22. Wilbar CW. Control of dengue in Hawaii. *Am J Pub Health.* 1947; 37:663–674. [PubMed: 20242031]

Dengue Neutralizing Antibody Titers in Seven Subjects Who Reported Experiencing Dengue Fever-like Illness (DFLIS) in 1943–1944

Table 1

Subject	Year of DFLI	Location of illness onset	IgG ELISA	Reciprocal titer					
				DENV-1 (Hawaii)	DENV-2 (DAR)	DENV-3 (H-87)	DENV-4 (TVP)	WNV (E101)	
1	1944	Guam	P	160	40	10	10	<10	
2	1944	New Guinea	P	≥320	160	10	20	<10	
3	1943	Hawaii	P	≥1280	40	10	160	<10	
4	1944	Guam	N	10	20	<10	10	10	
5	1943	Hawaii	N	<10	<10	<10	<10	<10	
6	1944	Hawaii	N	<10	<10	<10	<10	<10	
7	1944	Hawaii	P	≥320	40	10	20	<10	