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Original Article

Retrospective serological study on sequential dengue virus serotypes 1 to 4 epidemics in Tainan City, Taiwan, 1994 to 2000

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Background and Purpose: We previously reported the development of a non-structural protein NS1 serotype-specific immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) for dengue serodiagnosis and seroepidemiological study. This assay can be used to differentiate the immunologic status of individuals into naive, primary, or secondary dengue virus (DENV) infection and identify the DENV serotypes of primary infection. A retrospective study was conducted to investigate the serological responses of confirmed dengue cases infected during each of the sequential DENV-1 (August 1994 to February 1995), DENV-2 (August to December 1997), DENV-3 (August 1998 to January 1999), and DENV-4 (June to December 2000) epidemics in Tainan City, Taiwan.

Methods: 218 serum samples collected 1.1 to 7.2 years postinfection were analyzed by NS1 serotype-specific IgG ELISA together with corresponding acute and/or convalescent serum samples when available. The immunological status and the infecting DENV serotypes were determined for these individuals.

Results: High titers of dengue NS1 serotype-specific IgG antibody could be detected in serum samples. Differentiation of immunological status showed that 76.6% and 23.4% of cases had primary and secondary infections, respectively. A significant age-dependent increase in the rate of secondary infection was observed for those cases born before 1942. Notably, analysis of postinfection serum samples of 17 dengue hemorrhagic fever patients infected during the 1998 DENV-3 epidemic showed that 9 cases (53%) had primary infections.

Conclusions: Our data revealed that a majority of the population born after 1943 in Tainan City are naive to DENV infection and are at high risk of infection with all 4 DENV serotypes.

Key words: Dengue virus; Disease outbreaks; Enzyme-linked immunosorbent assay; Serologic tests; Viral non-structural proteins

Introduction

Dengue virus (DENV) is a mosquito-borne flavivirus and the most prevalent arbovirus in tropical and subtropical regions of the world. There are 4 distinct serotypes — DENV-1, DENV-2, DENV-3 and DENV-4. Infection induces a lifelong protective immunity to the homologous serotype, but only brief protection against heterologous serotypes. Clinical manifestations of dengue range from asymptomatic to nonspecific febrile illness, classic dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) [1,2]. The risk of developing DHF/DSS is increasing in patients secondarily infected with a different serotype through antibody-dependent enhancement [3,4]. Other factors postulated to be important in the pathogenesis of DHF include viral virulence, the host genetic background, cytokine storms, and the viral burden [5,6]. Differentiation of primary versus secondary or multiple DENV infection is critical in analyzing data for epidemiological, pathological, clinical and immunological studies [7-9]. Globally, the frequency

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of dengue epidemics has increased dramatically, with progressively larger epidemics every 3 to 5 years and the emergence DHF in Southeast Asia during the last 25 years [10].

Historically, epidemic dengue in Taiwan was first described in 1870. There were 3 large island-wide epidemics during the period of Japanese colonial rule, in the years 1915 to 1916, 1931, and 1942 to 1943, respectively. The 1981 DENV-2 epidemic in Islet of Liuchiu Township, Pingtung County, Taiwan, was the first dengue epidemic after World War II, and affected an estimated 80% of the inhabitants [11]. This was followed by an overwinter 1987 to 1988 DENV-1 outbreak, with dengue cases estimated at more than 100,000 in Kaohsiung City/County and Pingtung County. Since then, local outbreaks have been recorded almost every year in southern Taiwan [12-15]. A recent molecular epidemiological study of DENV infections in Taiwan strongly suggests that annual local outbreaks are caused by single or multiple imported DENV strains, which disappear with the ending of each outbreak [16]. Thus, dengue is not an endemic disease despite its annual occurrence brought about by constant importation of DENV strains from the neighboring Southeast Asian countries through close commercial links and air travel [17]. The non-endemic status of dengue epidemics is mainly due to the unique geographic location and climatic conditions in southern Taiwan, characterized by dry weather and periodic cold fronts bringing the temperature down to 10°C to 15°C in the wintertime.

For rapid diagnosis and seroepidemiological studies, we have developed a simple, sensitive and specific dengue NS1 serotype-specific immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) that can be used to differentiate the immunological status of individuals into naive, primary or secondary DENV infections, and identify the DENV serotypes of primary infection using convalescent phase or postinfection serum samples [18,19]. For better understanding of the current status of dengue epidemics in Taiwan, we initiated a pilot seroepidemiological study in the population of Liuchiu Township, Pingtung County, Taiwan. The results correlated very well with known epidemics, in that 73.1% of residents born between 1944 and 1980 were seropositive and an age-dependent increase in seroprevalence was observed [11,12]. The results also showed that dengue NS1 serotype-specific IgG ELISA could replace plaque reduction neutralization test for seroprevalence study.

We conducted a retrospective serological study on confirmed dengue cases infected during the sequential outbreaks from 1994 to 2000 in Tainan City. Tainan City is unique, with sequential DENV-1 (August 1994 to February 1995), DENV-2 (August to December 1997), DENV-3 (August 1998 to January 1999), and DENV-4 (June to December 2000) epidemics having occurred between 1994 and 2000 (Table 1). It is

Table 1. Summary of dengue epidemics between 1994 and 2000 in Tainan City

Period	DENV serotype	No. of confirmed cases	Frequency according to main administrative districts of epidemic	No. of dengue fever cases	No. of dengue hemorrhagic fever cases	No. of postinfection sera collected	No. of primary infections calculated from postinfection sera (%)	No. of secondary infections calculated from postinfection sera (%)
August 1994 to February 1995	DENV-1	43	Central (17) West (13) East (8)	42	1	25	20 (80.0)	5 (20.0)
August to December 1997	DENV-2	13	Central (12)	13	0	5	4 (80.0)	1 (20.0)
August 1998 to January 1999	DENV-3	138	Central (51) North (41) East (17)	115	23	111	81 (73.0)	30 (27.0)
June to December 2000	DENV-4	106	South (64) West (24) Central (13)	106	0	77	62 (80.5)	15 (19.5)
Total		300		276	24	218	167 (76.6)	51 (23.4)

Abbreviation: DENV = dengue virus

interesting to note that a relatively high proportion of dengue patients (23/138) infected in the 1998 DENV-3 outbreak were DHF cases [13]. These sequential epidemics provide a unique opportunity to study the persistence of dengue-specific antibody, the age-dependent immunological status (primary or secondary infection), and the association between disease severity, immunological status, virus strains and serotypes of the infecting virus. Here, we present the results from analyzing 218 postinfection serum samples of confirmed dengue cases infected during each of these sequential epidemics.

Methods

Study site

Tainan City is located in southern Taiwan and is the country's fourth largest city after Taipei, Kaohsiung and Taichung. It is situated at latitude 23°00' N and longitude 120°12' E (Fig. 1). It comprises an area of 175 km^2 and has a population of 750,000. Tainan City currently has 6 districts: Anping, Annan, East, West Central (merged from Central and West districts in 2004), South and North. In addition to the 4 regional, sequential dengue epidemics between 1994 and 2000 (Table 1), 3 island-wide large dengue epidemics that occurred between 1915 and 1916, in 1931 and from 1942 to 1943 were well documented.

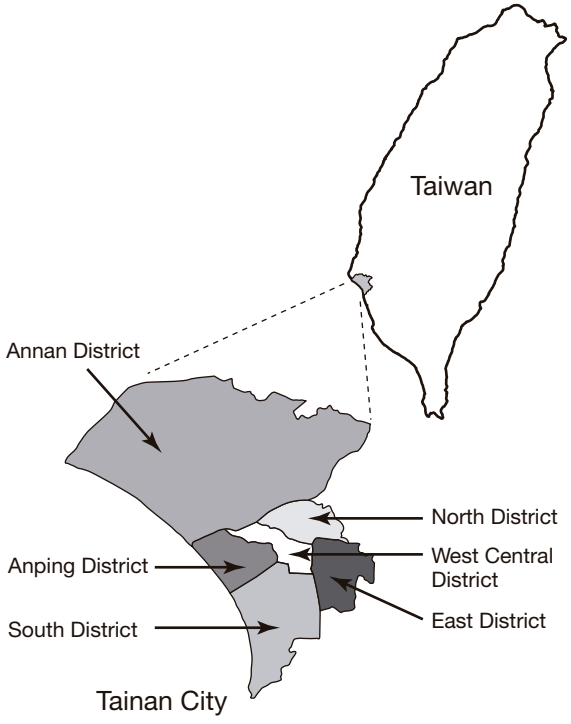

Fig. 1. Location of Tainan City, Taiwan, and its 6 administrative districts.

Human serum samples

A total of 218 postinfection serum samples were collected between November 22 and December 12, 2001 from volunteers of laboratory-confirmed dengue cases infected during each of the sequential DENV-1 (August 1994 to February 1995), DENV-2 (August to December 1997), DENV-3 (August 1998 to January 1999), and DENV-4 (June to December 2000) epidemics in Tainan City, Taiwan. Corresponding acute and/or convalescent serum samples were retrieved from the Dengue Serum Bank, Centers for Disease Control (CDC), Taiwan, and analyzed together with the postinfection serum samples when available. Laboratory-confirmed dengue cases were defined as febrile illness associated with the isolation of DENV, a positive reverse transcriptase-polymerase chain reaction (RT-PCR) test [20], or the detection of DENV-specific immunoglobulin M (IgM) and IgG antibodies (capture IgM and IgG ELISA) [19,21]. A clinical diagnosis of DHF was made according to the criteria of the World Health Organization [22]. Sera collected during 1 to 7 days after the onset of symptoms are referred to as acute-phase samples. Convalescent sera refer to specimens collected during days 8 to 30. Postinfection sera refer to specimens collected after 30 days of illness. Most of the postinfection sera used in this study were collected 1.1 to 7.2 years postinfection.

Cell culture and antigen preparation

The virus-infected culture supernatant was prepared as previously described, with slight modification [18]. Briefly, Vero cells at 8×10^6 were grown in 15 mL of Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco, Grand Island, NY, USA) containing 5% fetal calf serum in a T-75 flask. After incubation for 1 day in 5% CO_2 at 37°C, a monolayer was formed and infected with each of the 4 DENV serotype local strains, DENV-1 (8700828), DENV-2 (454009), DENV-3 (8700829) and DENV-4 (8700544) at a multiplicity of infection of 0.1. Virus culture supernatants were harvested 4 to 6 days later, inactivated by ultraviolet irradiation, divided into aliquots and stored at –80°C until use. The culture supernatants were used as the source of NS1 antigens for ELISA. To ensure that the titers of NS1 antigen used were optimal for each of the 4 DENV serotypes, a sandwich ELISA was set up to titrate the NS1 antigens in each batch

of the harvested supernatants [18]. These batches of culture supernatants showing saturated concentrations of NS1 antigen at 1:3 dilution were selected for NS1 serotype-specific IgG ELISA, in order that the equivalent NS1 antigens were captured in the wells through coated monoclonal antibodies. The control antigen was prepared by the same procedure from Vero cell culture without viral infection.

NS1 serotype-specific IgG ELISA

NS1 serotype-specific IgG ELISA was performed as previously described [17,18]. Briefly, each microtiter 8-well strip (Nunc-Immuno; Thermo Fisher Scientific, Rochester, USA) was coated with 5 μg/mL, 100 μL/well of monoclonal antibody D2/8-1 in 0.1 M Na_2CO_3/$NaHCO_3$ carbonate buffer (pH 9.5). After blocking and washing, the wells were incubated with 1:3 diluted NS1-containing culture supernatants of DENV-1, DENV-2, DENV-3, or DENV-4 infected Vero cells in phosphate-buffered saline + 0.05% Tween 20, 1% bovine serum albumin and 5% normal rabbit serum for 1 h at 37°C. After washing, serum samples were added at a 1:50 dilution and incubated for 1 h at 37°C. Goat anti-human IgG conjugated to alkaline phosphatase (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) was then added and the mixture incubated for 1 h at 37°C. The enzyme activity was developed with the addition of substrate *p*-nitrophenylphosphate and optical density (OD) was measured 30 min later at the dual wavelengths of 405 and 630 nm with a Dynatech MR700 microplate reader (Dynatech, NV, USA).

Data analysis of NS1 serotype-specific IgG ELISA

OD from culture supernatants of Vero cells with or without DENV infection was measured as test absorbance versus negative control for each ELISA sample. A positive result was determined by comparison to individual negative control. A positive sample was defined as having a test absorbance:negative control ratio of ≥2.0 and a negative sample was defined as having a ratio of <2.0. For those sera with positive NS1-specific IgG antibody response, NS1 serotyping was calculated as the ratio of the highest OD value to the second highest OD value read from the 4 DENV serotypes. Positive serotype specificity was defined as an OD ratio ≥1.2 and negative serotype specificity was defined as an OD ratio <1.2. Based on NS1 serotype-specific IgG ELISA, primary DENV infection was defined as negative NS1-specific IgG antibody response for sera collected between day 1 and 14 of illness, or positive dengue serotype specificity for sera collected ≥9 days of illness. Secondary DENV infection was defined as positive NS1-specific IgG antibody response for sera collected between day 1 and 8 of illness, or positive NS1-specific IgG antibody response and negative serotype specificity anytime after onset [19].

Results

Summary of dengue epidemics

Table 1 summarizes sequential DENV-1 to -4 epidemics in Tainan City, Taiwan. A total of 300 laboratory-confirmed cases were officially recorded, with 43 DENV-1, 13 DENV-2, 138 DENV-3, and 106 DENV-4 infections for each of the corresponding outbreaks. There was 1 DHF case in the 1994 DENV-1 outbreak and there were 23 DHF cases in the 1998 DENV-3 outbreak. The main administrative districts and case numbers in each of the 4 epidemics are shown. It should be noted that the Central and West districts were merged into West Central district in 2004. Due to limited overlapping of geographic location and the case numbers of these 4 epidemics, these dengue patients are believed to have been infected only once by DENV during the period from 1994 to 2000, in accordance with the official records of the CDC. Among these cases, 218 volunteers agreed to enroll in this study and postinfection sera were collected 1.1 to 7.2 years postinfection.

Postinfection serum samples were analyzed for NS1 serotype-specific IgG ELISA together with corresponding acute and/or convalescent serum samples, if available, from the Dengue Serum Bank, CDC. A total of 28 acute phase and 47 convalescent phase serum samples from 59 confirmed dengue cases were analyzed together with the 218 postinfection serum samples. Among these, 44 and 15 cases, respectively, were classified as primary and secondary infections from acute and/or convalescent serum samples, while 46 and 13 cases, respectively, were classified as primary and secondary infections from postinfection serum samples. The results show a 93.2% (55/59) correlation for the differentiation of primary and secondary infection status between acute and/or convalescent and postinfection serum samples. In order to establish whether DENV serotype identified by the NS1 serotype-specific IgG ELISA was in agreement with the corresponding serotypes determined by RT-PCR and/or virus isolation, we compared the results of these 2 assays. Among 52 acute phase serum samples positive

Table 2. Representative results showing non-structural protein 1 (NS1) serotype-specific immunoglobulin G (IgG) responses from postinfection serum samples of dengue patients infected between 1994 and 2000

Year of infection	DENV serotype	Type of dengue infection	Serum sample	Year of birth	Post-infection period (years)	NS1 serotype-specific IgG ELISA result (OD_{405})					
						DENV-1	DENV-2	DENV-3	DENV-4	NC	Ratio of NS1 serotyping[a]
1994	DENV-1	Primary	TN035	1937	7.16	1.81	0.69	0.62	0.29	0.20	2.63
			TN173	1947	7.00	1.58	0.46	0.58	0.30	0.16	2.72
		Secondary	TN032	1938	7.16	2.58	2.55	2.53	0.46	0.23	1.01
			TN216	1942	7.08	2.47	2.51	2.44	0.30	0.17	1.02
1997	DENV-2	Primary	TN036	1956	3.92	0.26	0.44	0.11	0.19	0.12	1.69
			TN157	1962	4.00	0.84	1.30	0.47	0.27	0.15	1.55
		Secondary	TN156	1944	4.00	0.83	0.88	0.55	0.39	0.32	1.06
1998	DENV-3	Primary	TN128	1983	3.00	1.57	1.00	2.97	0.61	0.23	1.89
			TN123	1954	3.00	1.56	1.57	3.37	0.84	0.12	2.15
		Secondary	TN161	1928	3.00	2.07	2.24	2.84	2.97	0.14	1.05
			TN148	1932	3.00	2.94	2.84	2.87	2.83	0.13	1.02
2000	DENV-4	Primary	TN067	1988	1.25	0.19	0.21	0.20	1.65	0.11	7.86
			TN111	1993	1.33	0.29	0.27	0.27	2.30	0.16	7.93
		Secondary	TN003	1942	1.16	2.36	1.54	2.15	1.63	0.19	1.10
			TN092	1943	1.16	2.45	2.09	2.51	1.65	0.14	1.02

Abbreviations: DENV = dengue virus; ELISA = enzyme-linked immunosorbent assay; OD = optical density; NC = negative control
[a]Ratios were calculated as the highest OD value to the second highest OD value read from the 4 DENV serotypes. Primary infection was defined as a ratio ≥1.2, while secondary infection was defined as a ratio <1.2.

by RT-PCR and/or virus isolation, 36 were found to be primary infection and serotyping was performed. There was 100% correlation in serotype specificity determined by NS1 serotype-specific IgG ELISA and RT-PCR and/or virus isolation. Table 2 shows the representative results from postinfection serum samples of dengue patients with primary or secondary infections. The results indicate that DENV serotypes identified by NS1 serotype-specific IgG ELISA are in accordance with the corresponding serotypes determined by RT-PCR and/or virus isolation. Analysis of immunological status showed that 76.6% and 23.4% of confirmed cases had primary and secondary infections, respectively. Similar ratios were found for dengue patients infected with DENV-1, DENV-2, DENV-3 and DENV-4 (Table 1).

Acute, convalescent, and postinfection NS1 serotype-specific IgG responses

Acute and/or convalescent serum samples available from the Dengue Serum Bank, CDC, corresponding to the acute, convalescent and postinfection phases were retrieved and analyzed together with postinfection serum samples. Table 3 shows the representative results from serum samples of dengue patients with primary DENV-3 or DENV-4 infections. The results show consistent NS1 serotype-specific IgG responses between acute, convalescent and postinfection serum samples with regard to immunologic status and identified DENV serotypes. It is interesting to note that NS1 serotype-specific IgG responses were usually higher in serum samples from the postinfection phase than in those from the convalescent phase for most of the serum samples tested. For most patients with secondary DENV infections, convalescent serum samples showed strong and complex non-serotype-specific NS1 IgG responses similar to the postinfection serum samples shown in Table 2.

Age-dependent increase in the rate of secondary dengue virus infection

In order to analyze the age-dependent variation in the immune status of infected individuals, postinfection serum samples were grouped into 15 age groups according to the year of birth. The results show an age-dependent increase in the rate of secondary infection, from 0% (born after 1990) to 100% (born between 1931 and 1935), then declining to 66.7% (born between 1926 and 1930) and 57.1% (born before 1926) [Fig. 2]. It is interesting to note a sharp increase of secondary infection rate to 75% in the age group born between 1941 and 1945, followed by another peak to

Table 3. Representative results showing non-structural protein 1 (NS1) serotype-specific immunoglobulin G (IgG) responses from acute, convalescent and postinfection serum samples of dengue patients with primary DENV-3 or DENV-4 infection in Tainan City

Patient no.	Time of serum sampling	NS1 serotype-specific IgG ELISA result (OD_{405})				
		DENV-1	DENV-2	DENV-3	DENV-4	NC
8701162 (DENV-3)	7 days	0.143	0.129	0.104	0.107	0.081
	27 days	0.609	0.610	1.146	0.441	0.122
	3 years	1.562	1.573	3.373	0.843	0.115
8701225 (DENV-3)	14 days	0.177	0.213	0.649	0.303	0.155
	43 days	0.207	0.192	0.748	0.217	0.147
	3 years	1.404	0.809	2.490	0.849	0.140
8701293 (DENV-3)	13 days	0.113	0.139	0.113	0.118	0.132
	121 days	0.560	0.543	1.025	0.298	0.136
	3 years	1.081	0.576	2.578	0.426	0.184
8900461 (DENV-4)	6 days	0.180	0.170	0.140	0.160	0.150
	13 days	0.210	0.200	0.180	0.770	0.150
	1.16 years	0.344	0.436	0.508	3.469	0.124
8900675 (DENV-4)	6 days	0.090	0.100	0.090	0.100	0.110
	28 days	0.230	0.310	0.330	1.300	0.110
	1.25 years	0.231	0.257	0.262	1.018	0.200
8900723 (DENV-4)	4 days	0.110	0.110	0.100	0.100	0.120
	16 days	0.170	0.210	0.230	0.980	0.120
	1.33 years	0.279	0.291	0.347	2.487	0.130
8900692 (DENV-4)	4 days	0.090	0.100	0.080	0.090	0.120
	16 days	0.130	0.300	0.240	1.190	0.130
	1.16 years	0.194	0.195	0.231	0.518	0.185

Abbreviations: DENV = dengue virus; ELISA = enzyme-linked immunosorbent assay; OD = optical density; NC = negative control

100% in the age group born between 1931 and 1935. These data are in accordance with the known dengue epidemics in that many old individuals might have been infected during the island-wide large outbreaks in Taiwan in 1931 and/or from 1942 to 1943, while most of young residents in Tainan City were naïve to DENV infection.

NS1 serotype-specific IgG responses in DENV-3-infected DHF

Among 23 DHF cases infected during the 1998 DENV-3 epidemics, 17 postinfection serum samples were available and analyzed for NS1 serotype-specific IgG responses (Table 4). Surprisingly, 9 (53%) vs 8 (47%) DHF cases were found to be primary and secondary infections, respectively. It is also interesting to note that all 9 primary cases were born after 1942, whereas all 8 secondary cases were born before 1942.

Discussion

For better understanding of the current status of dengue epidemics in Taiwan, we initiated serological, virological and epidemiological studies in high epidemic areas. A pilot investigation on healthy volunteers in Liuchiu Township, Pingtung County, Taiwan, during 1997 to 1998 showed that 93.6%, 87.5%, 73.1%, 24.6%, and 0.0% of serum samples were positive among residents born before 1931, between 1932 and 1941, between 1944 and 1980, between 1982 and 1986, and between 1989 and 1998, respectively [12]. More recently, a study in the Samin and Cianjhen districts of Kaohsiung City, a major epidemic focus of dengue, showed that 49.2% to 66.7%, 13.8% to 28.2%, and 3.9% to 13.3% of serum samples were seropositive for residents born before 1945, between 1946 and 1985, and between 1986 and 2000, respectively (unpublished data). The results provided close estimates of dengue prevalence rates of major dengue epidemics (1915 to 1916, 1931, 1942 to 1943, 1981, 1987 to 1988, and 2001 to 2002) affecting different areas of southern Taiwan during this century.

The unique sequential dengue epidemics of 4 DENV serotypes in Tainan City, Taiwan, during the period from 1994 to 2000 presented a unique opportunity to study the virological, immunological,

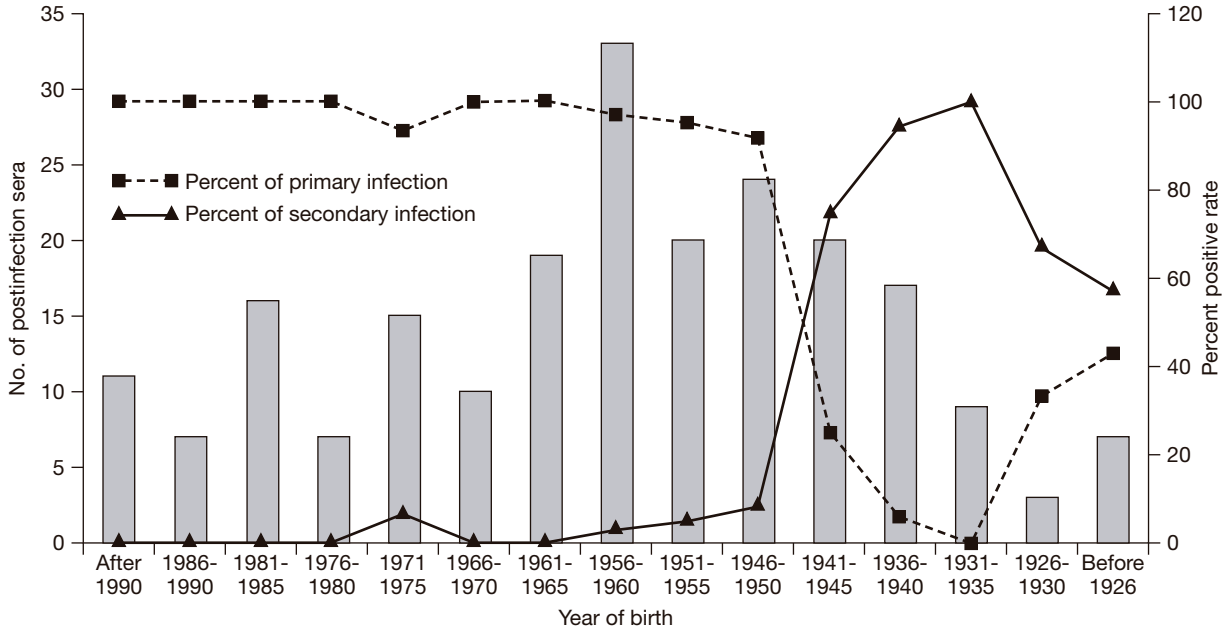

Fig. 2. Age distribution of confirmed dengue cases (columns) and the ratio of primary and secondary dengue virus infections (lines). A total of 218 postinfection serum samples collected after 1.1 to 7.2 years of infection from confirmed dengue cases infected during 1994 to 2000 sequential dengue epidemics in Tainan City were analyzed for non-structural protein 1 serotype-specific immunoglobulin G by enzyme-linked immunosorbent assay.

and pathological responses of DENV infections. In this study, we retrospectively tested confirmed dengue cases from November 22 to December 12, 2001. A total of 218 postinfection serum samples collected 1.1 to 7.2 years postinfection were analyzed for NS1 serotype-specific IgG ELISA together with corresponding acute and/or convalescent serum samples when available. The results demonstrated consistent NS1 serotype-specific IgG responses among acute, convalescent and postinfection serum samples of these patients of known DENV serotypes. It is interesting to note that the NS1 serotype-specific IgG responses were usually higher in serum samples from the postinfection phase than in those from the convalescent phase. It is possible that NS1-specific IgG antibody does not reach its peak in the convalescent phase.

Analysis of immunological status by age group showed an age-dependent increase in the rate of secondary infection from 0% (born after 1990) to 100% (born between 1931 and 1935). Further analysis showed a sharp increase in the rate of secondary dengue infection, to 75% of cases, in the age group born between 1941 and 1945, followed by another peak of 100% of cases in the age group born between 1931 and 1935. These data were consistent with the known 1942 to 1943 island-wide large outbreaks in Taiwan. Indeed, Tainan City was the major city affected during the dengue epidemic from 1942 to 1943. In contrast, the majority of younger individuals in Tainan City were naive to DENV infection due to limited dengue transmission after World War II. The reason for the decline in the rate of secondary infection for those individuals born before 1930 is not known at present, and could not be investigated further in this study, due to the limited sample size. We are currently conducting a seroepidemiological study in Tainan City to address this issue.

The 1998 DENV-3 outbreak was uncommon in that a relatively high proportion of dengue patients (23/138) were DHF cases. Moreover, in contrast to most dengue epidemics in Taiwan, the DHF cases were highly correlated with advanced age and secondary infection (unpublished data). As shown in Table 4, there is excellent correlation between the age distribution and immunological status of these DHF cases. All 9 DHF patients born after 1942 were primary infections, while the other 8 DHF patients born before 1942 were secondary infections. NS1 serotype-specific IgG ELISA confirmed that all 9 primary DHF cases were infected with DENV-3. The relatively high proportion of DHF cases observed suggests that this DENV strain might be more virulent. Further analyses of gene sequences [23] and virological characterizations of various DENV-3 strains might provide

Table 4. Non-structural protein 1 (NS1) serotype-specific immunoglobulin G (IgG) responses from postinfection serum samples of 17 DENV-3-infected dengue hemorrhagic fever cases

Patient no.	Postinfection sera (3 years)	Gender	Year of birth	Infection	NS1 serotype-specific IgG ELISA result (OD_{405})					
					DENV-1	DENV-2	DENV-3	DENV-4	NC	Ratio of NS1 serotyping[a]
8701142	TN197	Male	1978	Primary	1.19	0.87	2.67	0.63	0.16	2.24
8701291	TN057	Female	1975	Primary	1.88	1.66	3.17	1.32	0.19	1.69
8701202	TN193	Male	1971	Primary	1.05	0.75	2.60	0.66	0.22	2.48
8701393	TN135	Male	1969	Primary	2.22	1.59	3.09	1.64	0.18	1.39
8701312	TN191	Male	1967	Primary	1.05	0.71	2.25	0.37	0.12	2.14
8701203	TN211	Female	1960	Primary	1.59	1.07	3.07	0.68	0.13	1.93
8701289	TN027	Male	1959	Primary	0.32	0.27	0.84	0.24	0.19	2.63
8701438	TN204	Female	1956	Primary	1.59	1.14	2.95	0.75	0.14	1.86
8701418	TN115	Female	1949	Primary	1.75	1.39	2.63	1.00	0.20	1.50
8701318	TN040	Female	1941	Secondary	2.56	2.30	2.15	0.85	0.18	1.11
8701158	TN210	Female	1938	Secondary	1.95	2.03	1.92	0.57	0.40	1.04
8701285	TN170	Male	1935	Secondary	2.73	2.38	2.04	1.14	0.15	1.15
8701336	TN217	Male	1935	Secondary	2.39	2.23	1.97	0.48	0.36	1.07
8701201	TN212	Female	1935	Secondary	2.69	1.88	2.26	2.04	0.14	1.19
8701244	TN039	Female	1933	Secondary	3.37	3.24	3.17	2.28	0.19	1.04
8701096	TN161	Male	1928	Secondary	2.07	2.24	2.84	2.97	0.14	1.05
8701364	TN018	Male	1925	Secondary	2.64	3.18	2.33	1.74	0.20	1.19

Abbreviations: DENV = dengue virus; ELISA = enzyme-linked immunosorbent assay; OD = optical density; NC = negative control
[a]Ratios were calculated as the highest OD value to the second highest OD value read from the 4 dengue virus serotypes. Primary infection was defined as a ratio ≥1.2, while secondary infection was defined as a ratio <1.2.

information important in the understanding of DHF pathogenesis in primary DENV infection. Recently, Anantapreecha and colleagues reported that almost all of the DHF cases in Thailand caused by DENV-2 and DENV-4 were secondary DENV infections, and one-fifth of DHF cases caused by DENV-1 and DENV-3 were primary DENV infections [24]. These results suggest that DENV-1 and DENV-3 strains are more virulent than DENV-2 and DENV-4, and more likely to cause DHF in primary infection.

In conclusion, this retrospective serological study on sequential DENV epidemics in Tainan City, Taiwan, during the period 1994 to 2000 demonstrated that high titers of dengue NS1 serotype-specific IgG antibody can be detected in postinfection serum samples and that this assay can be reliably used for serodiagnosis and seroepidemiological study. Our data also revealed that the majority of the population of Tainan born after 1943 were naïve to DENV infection and at high risk of infection with all 4 DENV serotypes. This is in contrast to the Samin and Cianjhen districts of Kaohsiung City, Taiwan, where about 25% of residents were dengue-seropositive due to previous large DENV-1 (1987 to 1988) and DENV-2 (2001 to 2002) epidemics, and that the population was at high risk of DENV-3 and DENV-4 infections. Future seroepidemiological study on various areas of major epidemic foci should provide information important for developing effective strategies for dengue control measures in Taiwan.

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