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Detection of Immune-Complex-Dissociated Nonstructural-1 Antigen in Patients with Acute Dengue Virus Infections

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Accurate and timely diagnosis of dengue virus (DEN) infections is essential for the differential diagnosis of patients with febrile illness and hemorrhagic fever. In the present study, the diagnostic value of a newly developed immune-complex dissociated nonstructural-1 (NS-1) antigen dot blot immunoassay (DBI) was compared to a commercially available DEN antigen detection kit (denKEY Blue kit; Globio Co., Beverly, Mass.) and a reverse transcription-PCR (RT-PCR) kit. Serial serum or plasma samples (n = 181) obtained from 55 acute DEN-infected patients were used. In samples obtained from 32 of these 55 DEN-infected patients, viral RNA could be detected by RT-PCR. DEN antigen was detected in only 10 of these 55 patient samples by using the denKEY kit. When these samples were treated with acid to release the immune-complex-associated NS-1 antigen for detection by DBI, 43 of these 55 patients were found to be positive for DEN NS-1 antigen. In nondissociated samples, 22 of these patients were found to be positive by the DBI. In the presence of DEN-specific immunoglobulin M antibodies, both viral RNA and DEN (NS-1) antigen could be detected. The number of positive samples identified by RT-PCR and DBI from these patients with primary DEN infections varied between 28 and 78%. In secondary DEN infections, the number of samples that tested positive by the DBI after immune-complex dissociation (DIS-DBI) was 25% higher than the number of samples that tested positive by RT-PCR and was 35% higher than that determined by nondissociated antigen (NDIS-DBI) detection. We conclude that the denKEY kit has limited diagnostic value for acute DEN infections compared to the RT-PCR and the NDIS-DBI and DIS-DBI methods. We clearly demonstrate that in secondary DEN infections the dissociation of NS-1 immune complexes is essential for early diagnosis of DEN infections.

Dengue virus (DEN) is one of the most widespread mosquito-borne human pathogens worldwide, accounting for more than 50 million infections per year (10). Mosquitoes of the *Aedes* species are responsible for transmitting the four serotypes of DEN (DEN1 to DEN4) to humans. Infection with DEN may be asymptomatic or may cause a variety of symptoms ranging from mild dengue fever (DF) to the more severe form of dengue hemorrhagic fever (DHF) with or without shock (dengue shock syndrome [DSS]) (17). In areas where DEN is endemic, DHF has become an increasingly important cause of pediatric morbidity and mortality since it was first described half a century ago (17). Accurate diagnosis of DEN infections is therefore essential.

The diagnostic methods of choice for the identification of DEN infections have been the plaque reduction neutralization assay and/or virus isolation from patient serum samples by using mosquito cell lines (17, 18). However, both of these assays are laborious to perform and a period of at least 7 days is required to obtain accurate diagnostic results using them. Recently, several enzyme-linked immunosorbent assays (ELISAs) have become commercially available for the detection of DEN-specific antibodies of different isotypes (6, 7). However, DEN serology is not virus specific but shows a high amount of cross-reactivity with other flaviviruses (8). Detection of viral RNA in serum samples from acute-phase DEN-infected patients by using a reverse transcription-PCR (RT-PCR) has been described and is a valuable tool for both diagnosis of DEN infections and the identification of the viral serotype (11). RT-PCR provides an accurate diagnosis for DEN infections during the early stages of DEN illness, even in the presence of DEN-specific immunoglobulin M (IgM) antibodies (2). The RT-PCR is, however, relatively expensive to use as a routine diagnostic test and requires specialized laboratory equipment and trained personnel. In addition, the storage of the serum samples at −70°C that is essential for RT-PCR in order to maintain viral RNA in optimal conditions is not feasible in many areas where DEN is endemic. As an alternative, the detection of viral antigens has been proposed (19) and a suitable ELISA (12) can be performed with patient serum samples that have been stored at 4°C. A simplified immunoassay for the detection of DEN antigen in patient samples with a sensitivity and specificity comparable to the RT-PCR would therefore be highly desirable. The DEN nonstructural-1 (NS-1) protein has been identified as either an intracellular membrane-associated protein or a soluble extracellular protein (3). Since high concentrations of the NS-1 protein were found in blood samples of patients obtained during the early acute phase of both primary and secondary DEN infections and for up to 9 days after the onset of symptoms (1),

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DEN NS-1 detection assays are likely to be valuable diagnostic tools. Anti-NS-1 antibodies were rarely detected by immunoblot assays in samples from patients with primary DEN infections, but these antibodies were detected much more frequently in patients with secondary DEN infections, in particular among patients from areas where DHF and DSS is more frequent (such as Indonesia), compared to patients from areas where DHF and DSS is not so common (such as the Caribbean) (10).

Dissociation of antibody-antigen (Ab-Ag) immune complexes has proven to be important for the early diagnosis of several blood-borne viruses such as human immunodeficiency virus and both hepatitis B and C viruses (13, 14, 16). This technique has not, however, been applied in the diagnosis of flavivirus infections and in particular for DEN infections.

In the present study, we assessed the diagnostic value of dissociated and nondissociated DEN NS-1 Ab-Ag immune complexes in samples from primary and secondary DEN patients by using a dot blot immunoassay (DBI). The results were compared to a commercially available DEN antigen detection kit (denKEY Blue; Globio Co., Beverly, Mass.) and DEN-specific RT-PCR.

MATERIALS AND METHODS

Serum and plasma samples. Serial serum and plasma samples were collected from January 2000 to March 2002 from 55 patients with acute DEN infections and stored in aliquots at −80°C until use. The diagnosis of the patients was based on the clinical and serological criteria set by the World Health Organization (17). Thirty-nine patients were from Indonesia, and plasma samples ($n = 156$) were obtained upon admission to the hospital (D0) and on days 1, 2, and 7 postadmission (D1, D2, and D7, respectively). All of these Indonesian patients were children; 20 of them were suffering from DSS. Twenty-five serum samples were obtained from 16 patients from the Dutch Antilles. From nine of these patients, serum samples were obtained upon presentation to their treating physician (D0) and also 2 weeks later (D14), whereas from the remaining seven patients only one serum sample was available obtained upon presentation to the clinic (D0). The patients from the Antilles were all adults with DF. In all, 18 patients had primary DEN infections and 37 patients had secondary DEN infections based on their serological profiles (17). Sixteen serum samples were obtained from healthy Dutch blood donors with no evidence of current or previous DEN infection (as defined by the absence of DEN specific IgM and/or IgG antibodies) served as the negative controls.

IgM and IgG ELISA. A commercially available kit (Focus Technologies, Cypress, Calif.) was used for the detection of DEN-specific IgM and IgG antibodies in the samples and performed according to the procedures described by the manufacturer. The sensitivity and specificity of the assays has been described elsewhere (6).

Dot blot assay. Patient serum or plasma samples were diluted either 1:3 in phosphate-buffered saline (PBS) (nondissociated samples) or 1:2 in dissociation buffer (1.5 M glycine [pH 2.8]). Ab-Ag immune complexes were dissociated for 1 h at 37°C, and the reaction was stopped by the addition of 1 volume of neutralization buffer (1.5 M Tris-HCl [pH 9.7]) to achieve an end dilution of 1:3 for the sample. The samples were then spotted onto nitrocellulose membranes (High Bond-P membranes; Amersham Biosciences, Little Chalfont, United Kingdom). After the samples were spotted onto nitrocellulose, the membranes were air dried, treated with methanol containing 0.3% H_2O_2, washed with distilled water and PBS, and blocked overnight at 4°C with 5% skimmed milk (ELK Campina, Eindhoven, The Netherlands) in PBS containing 0.1% Tween 20 (PBS-T). The blots were then washed with PBS-T and incubated for 30 min at room temperature with monoclonal antibody 3D1.4, which defines the LX1 epitope on the NS-1 proteins of each DEN serotype (4), diluted in PBS-T buffer containing 5% skimmed milk, 5% normal goat serum (ICN Biochemicals, Inc., Cleveland, Ohio), 5% fetal bovine serum (ICN Biochemicals, Inc.), and 20% normal human serum (blot buffer). The blots were then washed thoroughly for 30 min with PBS-T and incubated for 30 min at room temperature with goat anti-mouse horseradish peroxidase conjugate (Dako, Glostrup, Denmark) diluted 1:10,000 in blot buffer. The blots were washed, incubated in PBS for 5 min, and further processed with the ECL substrate system (ECL; Amersham Biosciences) according to the procedures described by the manufacturer. Films (Kodak X-Omat AR) were exposed for 5 min before development.

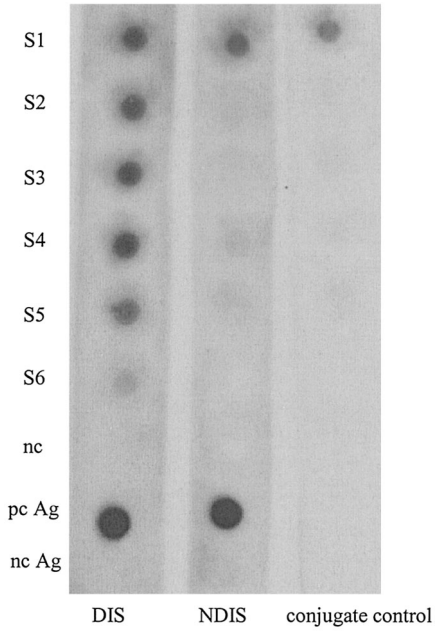

FIG. 1. Detection of DEN NS-1 antigen with the DBI in serum or plasma samples after dissociation (DIS) of immune complexes or without dissociation (NDIS) of immune complexes. pc Ag, positive control (DEN antigen); nc Ag, negative control antigen; nc, negative control human serum. Acetone-extracted positive and negative antigen controls were prepared as previously described (7).

DEN antigen detection ELISA. The presence of DEN antigen in the serum and plasma samples was measured by using a newly described, commercially available kit (Globio Co.) based on an ELISA method according to the procedures of the manufacturer. Briefly, diluted samples are incubated for 2 h in microtiter wells, followed by a 1-h incubation step with a DEN-specific antibody. When DEN antigen was present in the serum samples, it was bound by the detecting antibody, and the Ab-Ag immune complexes were then detected after the addition of an enzyme-labeled conjugate and subsequent substrate reaction.

Detection of viral RNA. DEN RNA was isolated from 200 μl of patients' serum or plasma by using the High-Pure RNA extraction kit (Roche, Mannheim, Germany) according to the procedure described by the manufacturer. DEN serotype-specific reverse transcriptase, followed by PCR (i.e., RT-PCR) was performed as described previously (11, 15). Patient samples were considered positive when a band of the correct size (DEN1, 482 bp; DEN2, 119 bp; DEN3, 290 bp; and DEN4, 392 bp) was observed by using a UV illuminator after 2% agarose gel electrophoresis and subsequent staining with ethidium bromide.

Calculations. For the determination of DEN-specific IgM and IgG antibody ratios, the cutoff values provided by the manufacturer were used. Samples were considered positive with the dot blot assay when a clear spot could be visualized on films, in the absence of signal in the conjugate control. Readings of the dot blot assays (i.e., the DBI) were performed by two independent observers. Samples were considered positive by using the denKEY kit, based on the criteria of the manufacturer, when an optical density value of >0.150 was obtained.

RESULTS

Detection of DEN NS-1 antigen in dissociated and nondissociated samples. The DEN NS-1 detection sensitivity of the DBI was higher when the patient samples were treated with acid to dissociate their Ab-Ag immune complexes, as shown in Fig. 1. Of the 181 samples tested by the DBI, DEN NS-1 antigen could be detected in only 32 of them without Ab-Ag dissociation (NDIS-DBI), whereas this antigen could be de-

TABLE 1. Detection of DEN NS-1 antigen and viral RNA in patients with primary or secondary DEN infections

Assay[a]	No. of positive samples (% of total)		
	Primary infections (n = 18)	Secondary infections (n = 37)	Total (n = 55)
denKEY kit	5 (28)	5 (13)	10 (18)
RT-PCR	12 (67)	20 (54)	32 (58)
NDIS-DBI	12 (61)	10 (27)	22 (40)
DIS-DBI	14 (77)	29 (78)	43 (78)

[a] NDIS, samples not treated with acid for dissociation of Ab-Ag immune complexes; DIS, samples treated for dissociation of Ab-Ag immune complexes.

TABLE 2. Detection of DEN NS-1 antigen by using NDIS-DBI and DIS-DBI in samples obtained from Indonesian and Antillean patients with primary and secondary DEN infections[a]

Infection type	No. of positive Indonesian samples (n = 39)[b] (% of total)		No. of positive Antillean samples (n = 16)[c] (% of total)	
	NDIS	DIS	NDIS	DIS
Primary	7 (64)	10 (91)	5 (72)	4 (57)
Secondary	6 (22)	26 (93)	4 (44)	3 (33)

[a] See Table 1, footnote *a*.
[b] Eleven primary and twenty-eight secondary infections.
[c] Seven primary and nine secondary infections.

tected in 113 of these samples after acid treatment (DIS-DBI). Of the 32 NDIS-DBI-positive samples, 24 were also positive in the DIS-DBI.

Detection of DEN antigen in the presence of DEN-specific IgM antibodies. The DEN antigen detection level of the denKEY kit was low, since only 10 of the 181 patient samples had optical density values higher than the cutoff value. Nine of the ten denKEY antigen-positive patients were positive on day 0 after admission to the hospital, whereas one patient was DEN antigen positive on day 7 after admission to the hospital. The denKEY kit identified DEN antigen in five patients with primary DEN infections and five patients with secondary DEN infections (Table 1). There was no correlation between the very low DEN antigen detection levels in these patient samples obtained with this assay and the presence of DEN-specific IgM antibodies in these samples (Fig. 2), although most of these DEN-antigen positive samples had relatively low or negative titers to IgM antibodies.

The DEN antigen detection level of the DBI was significantly higher than that obtained by using the denKEY kit, especially when samples were treated to dissociate immune complexes (DIS-DBI). In 22 of the 55 DEN-infected patients, DEN NS-1 antigen could be detected by the DBI without the dissociation step being necessary. Of these patients, 12 were experiencing primary DEN infections and 10 were experiencing secondary DEN infection (Table 1). When the patient samples were dissociated, 43 of them were found to be positive for DEN NS-1 antigen; 14 of these had primary DEN infections and 29 had secondary DEN infections (Table 1). Detection of DEN NS-1 antigen was not influenced by the presence of DEN-specific IgM antibodies when evaluated with NDIS-DBI or DIS-DBI (Fig. 2).

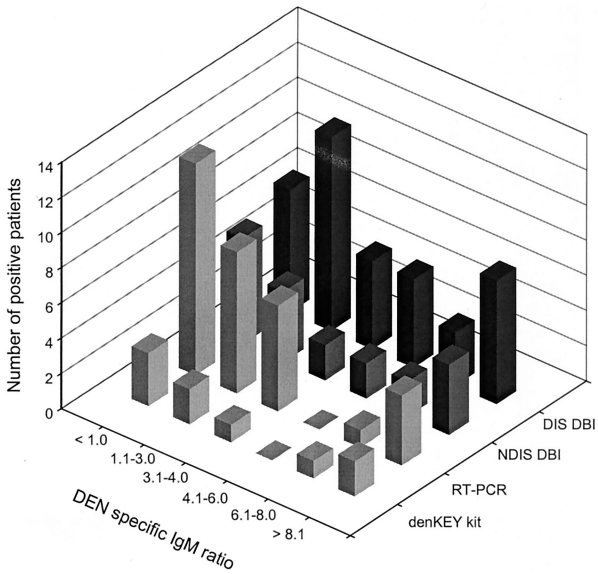

FIG. 2. Detection of DEN NS-1 antigen and viral RNA with different assays compared to the levels of DEN-specific IgM antibodies in samples obtained from patients with acute DEN infection. The z axis represents the number of positive patients with each assay from samples obtained on day 0.

Detection of DEN NS-1 antigen in patients' samples from different areas where DEN is endemic. The DEN NS-1 antigen detection level determined by NDIS-DBI in samples from patients with primary DEN infection was similar for patients from Indonesia (64%) or from the Dutch Antilles (72%). The DEN NS-1 antigen detection level was increased in samples from primary-DEN-infection Indonesian patients (91%) but slightly reduced in samples from primary-DEN-infection Antillean patients (57%) when tested by DIS-DBI (Table 2). In samples obtained from patients with secondary DEN infections, the DEN NS-1 antigen detection levels were low among Antillean samples: 44% with the NDIS-DBI and 33% with the DIS-DBI. In contrast, the detection level of DEN NS-1 antigen in samples from Indonesian patients with secondary DEN infections increased dramatically from 22% with the NDIS-DBI to 93% with the DIS-DBI (Table 2).

Comparison of DEN type-specific RT-PCR and DEN antigen detection assays. A comparison of DEN antigen and viral RNA detection with the denKEY kit, the DBI, and RT-PCR in serially collected samples obtained from patients who lived in areas where DEN is endemic is shown in Fig. 3. Using the RT-PCR, DEN RNA was detected in 32 of the 55 patients with acute DEN infections. Eight patients were infected with DEN1 (seven Indonesian patients and one Antillean patient), four patients were infected with DEN2 (all Indonesian patients), nineteen patients were infected with DEN3 (four Indonesian and fifteen Antillean patients), and one patient from Indonesia was infected with DEN4 (Table 3). All but one of the 32 RT-PCR-positive patients were positive on day 0 (the day of first presentation to the clinician), whereas five patients remained positive for viral RNA on days 1 and 2 postadmission to the hospital. Only one patient was positive by RT-PCR on day 7 after admission to the hospital (Fig. 3). Of the 16 RT-PCR-positive Indonesian patients for whom clinical records were available, 6 patients had DSS, while 10 had DHF.

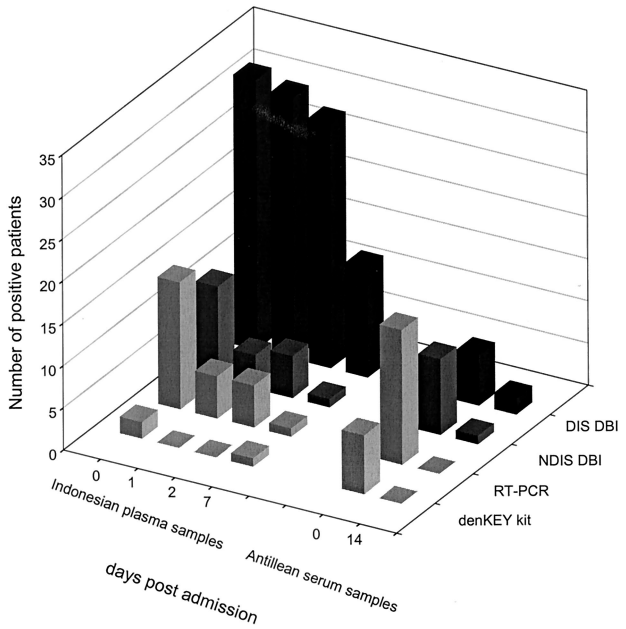

FIG. 3. Kinetics of DEN NS-1 antigen and viral RNA detected with the different assays on different time points after the onset of illness. Day 0 is the day of presentation to the clinic and represents days 3 to 6 after the onset of illness.

Using the denKEY kit, DEN antigen was detected in 1 of the 8 DEN1-infected patients, in none of the DEN2-infected patients, in 8 of the 19 DEN3-infected patients, and in the single DEN4-infected patient (serotypes were determined by RT-PCR). The one patient who was found to be positive by the RT-PCR on day 7 after admission to the hospital was also found to be positive with the denKEY kit (Table 3).

Using the NDIS-DBI, DEN NS-1 antigen was detected in 6 of the 8 DEN1-infected patients, in 1 of the 4 DEN2-infected patients, in 10 of the 19 DEN3-infected patients, and in the single DEN4-infected patient. After dissociation of the patients' immune complexes (DIS-DBI), DEN NS-1 antigen was detected in 6 of the 8 DEN1-infected patients, in all 4 DEN2-infected patients, in 11 of the 19 DEN3-infected patients, and in the DEN4-infected patient (Table 3). In addition, DEN NS-1 antigen was detected by NDIS and DIS-DBI in 19 patients, who were found to be negative by the RT-PCR for the presence of viral RNA (Fig. 3).

TABLE 3. Comparison of methods for detection of viral antigen (denKEY antigen kit and DBI) versus viral RNA (RT-PCR) in samples obtained from 55 DEN-infected patients

Method	No. of samples positive by RT-PCR (n = number of positive patients) for:				
	DEN1 (n = 8)	DEN2 (n = 4)	DEN3 (n = 19)	DEN4 (n = 1)	Total (n = 32)
denKEY kit	1	0	8	1	10
NDIS-DBI	6	1	10	1	18
DIS-DBI	6	4	11	1	22
Total NDIS and DIS-DBI	8	4	14	1	27

[a] NDIS, nondissociated samples; DIS, Ab-Ag immune complex-dissociated samples.

TABLE 4. Overview of the results for the detection of DEN NS-1 antigen and viral RNA in 181 samples from DEN-infected patients by using the denKEY kit, the DBI, and RT-PCR

Method	Score[a]	No. of positive samples as determined by:		
		RT-PCR	DEN NS-1 DBI[b]	
			NDIS	DIS
denKEY kit	++	10	9	6
	+−	0	1	4
	−+	32	24	107
	−−	139	147	64
NDIS-DBI	++	23		25
	+−	9		7
	−+	19		88
	−−	130		61
DIS-DBI	++	28	25	
	+−	85	88	
	−+	14	7	
	−−	54	61	

[a] +, sample scored positive in the respective test; −, sample scored negative in the respective test.
[b] NDIS, nondissociated samples; DIS, dissociated samples.

Overall comparison. Table 4 summarizes the overall comparison of the DEN (NS-1) antigen detection assays and the RT-PCR for the 181 serum and plasma samples tested. A total of 42 serum samples tested positive for the presence of viral RNA by using the RT-PCR; of these, only 10 (23%) tested positive with the denKEY kit, 23 (55%) tested positive with the NDIS-DBI, and 28 (67%) tested positive with the DIS-DBI. All of the 139 serum samples that tested negative with the RT-PCR were also negative with the denKEY kit; 130 (93%) tested negative with the NDIS-DBI, whereas only 54 (39%) tested negative with the DIS-DBI (Table 4).

DISCUSSION

Routine diagnosis of DEN infections is mainly based on serological assays for the detection of DEN-specific IgM and IgG antibodies. Detecting DEN antigen in serum and plasma samples with a simple, rapid, and easy-to-perform assay, such as an ELISA or a dot blot assay with a sensitivity and specificity similar to that of the RT-PCR, would be an excellent alternative for routine diagnosis of acute DEN infections.

In the present study we describe a DBI for the detection of the DEN NS-1 antigen, since high concentrations of this antigen were previously shown to be present in patient samples obtained during the early acute phase of either primary or secondary DEN infections (1, 19). Since antibodies to the DEN NS-1 protein are generated, particularly during secondary DEN infections in patients from Indonesia (an area where DHF and/or DSS is common) compared to the DEN infections in patients from Puerto Rico (an area where DHF and/or DSS is less common) (9), we also chose to compare the relative sensitivities of these assays in both primary and secondary DEN infections in patients from Indonesia and the Dutch Antilles before and after dissociation of the immune complexes. The predominant DEN serotype circulating during the outbreaks in Indonesia and the Dutch Antilles was DEN3 (5).

All patients from Indonesia were children with DHF and/or DSS, whereas the patients from the Antilles were all adults with symptoms of DF. For our assay, we used a monoclonal antibody (3D1.4) that detects the minimal peptide sequence YSWKTWG, which is present in the NS-1 proteins of three DEN serotypes. The potentially higher specificity of this monoclonal antibody for the NS-1 protein of DEN1 was not, however, reflected in the relative NS-1 detection sensitivities for the other DEN serotypes (4). This monoclonal antibody was more reactive with the corresponding sequence from the NS-1 protein of DEN1, which contained a single amino acid substitution (YSWKSWG) (4).

In the present study, we demonstrated that nondissociated DEN NS-1 antigen was detected in 61% of DEN primary cases compared to 27% detection of the secondary cases (Table 1). Furthermore, we demonstrated that nondissociated DEN NS-1 antigen was detected in the majority of the patients with primary DEN infection from Indonesia and the Dutch Antilles but could be further increased in the Indonesian patient samples with the DIS-DBI. Dissociation of DEN NS-1 Ab-Ag immune complexes proved to be essential for the detection of DEN NS-1 antigen in serum and plasma samples, particularly among samples obtained from Indonesian patients with secondary DEN infections since the sensitivity of the DBI increased dramatically (up to 93%) when these dissociated samples were tested. These results are in agreement with those of a previous study in which it was demonstrated that anti-NS-1 antibodies are mainly generated during secondary DEN infections in patients from Indonesia (where DHF and/or DSS is more common) than in patients from the Caribbean (where DHF and/or DSS is not common) (10). These preexisting anti-NS-1 antibodies possibly result from the formation of Ab-Ag immune complexes and therefore reduce the sensitivity of the DEN NS-1 antigen detection assay, in particular when the NDIS-DBI is used.

The sensitivity of the denKEY kit was very poor, since only 10 samples tested positive for the presence of DEN antigen with this assay. However, both the denKEY kit and DBI of nondissociated samples detected DEN NS-1 antigen with greater sensitivity in samples from primary infections, whereas DBI of dissociated samples detected DEN NS-1 antigen with the same sensitivity in samples from either primary or secondary infections. In a previous study it was demonstrated that DEN viral RNA could be detected in the presence of DEN-specific IgM antibodies in serum or plasma samples of DEN-infected patients (2). In our study we confirmed these results and, furthermore, we demonstrated that DEN NS-1 antigen could also be detected in the presence or absence of DEN-specific IgM antibodies. However, the number of NS-1 antigen-positive patients in the presence of IgM was significantly higher in dissociated samples at different levels of IgM antibodies (Fig. 2).

We also studied the number of DEN antigen-positive patients at several time points after infection. Recently, it was demonstrated that DEN NS-1 antigen could be detected between days 0 and 9, with a slight peak on day 4 after the onset of illness (1). Our findings are in agreement with this previous study since we demonstrated that the majority of positive samples for DEN NS-1 antigen were obtained in patients between days 0 and 2 after admission to the hospital (i.e., 3 to 6 days after the onset of symptoms). Furthermore, we were able to detect the DEN NS-1 antigen with the DBI during the early convalescent phase of DEN illness in 15 of the 55 DEN patients (Fig. 3). Viral antigens circulate in patients' blood for longer periods than viral RNA, and we were therefore able to detect DEN NS-1 antigen in many patient samples in which viral RNA was not detectable (i.e., RT-PCR negative). The results we obtained with the RT-PCR showed that the highest percentage of positive samples was detected on day 0 in both cohorts (97%). However, on days 1 to 7 RT-PCR-positive results were also obtained.

The specificity of the DEN NS-1 antigen detection DBI did not seem to be influenced by the infecting DEN serotype. In contrast, the denKEY kit seems to detect DEN 3 with a higher sensitivity (43% of the DEN3-infected patients were also found to be positive with the denKEY kit) than the other three serotypes. However, it is difficult to draw any reliable conclusions about the serotype specificity of the assays. Our sample size of DEN-positive patients with the RT-PCR was rather small, and there was not a similar incidence of each of the four DEN serotypes among our patients (e.g., we were able to detect DEN4 viral RNA in only one patient). We could not, therefore, perform a statistical analysis on the serotype specificity of the DEN NS-1 antigen detection assays in more detail. Furthermore, based on the clinical data available, we were not able to find any significant differences associated with disease severity and the presence of DEN NS-1 antigens in acute- and/or convalescent-phase samples.

In conclusion, we demonstrated here that the combination of NS-1 antigen detection by DBI in both nondissociated and dissociated serum and plasma samples from primary and secondary cases of DEN infection results in the highest number of DEN antigen-positive patients versus the RT-PCR and the denKEY kit. We also showed that dissociation of immune complexes is essential for the detection of DEN NS-1 protein in secondary cases of DEN infection. This newly developed method also offers the opportunity for rapid identification of dengue infections among many other flavivirus circulating in areas where DEN is endemic and causing similar clinical symptoms; this method also overcomes several problems associated with the RT-PCR approach. For example, patient serum or plasma samples could be adequately stored at 4°C for subsequent testing with this assay since the DEN NS-1 antigen is more stable than viral RNA, and the problems due to contamination of RT-PCR products are also not an issue in antigen detection assays. In addition, DBI is rapid, easy to perform, and can be carried out in less-well-equipped laboratory settings. The high sensitivity of the DBI in detecting DEN NS-1 antigen in samples from acute primary or secondary DEN infection patients make it a valuable diagnostic tool and a good alternative to the RT-PCR. In contrast, we found the denKEY kit to be unsuitable for DEN virus diagnosis due to its low sensitivity.

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