Sera of patients with systemic lupus erythematosus cross-neutralized dengue viruses

Running Title: SLE serum cross-neutralized dengue virus

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ABSTRACT

Dengue is the most prevalent mosquito-borne disease in the Southeast Asia, where systemic lupus erythematosus (SLE) occurrence is at approximately 30 to 53 per 100,000. Severe dengue, however, is rarely reported among patients with SLE. Here, we investigated if sera of patients with SLE would cross-neutralize dengue virus (DENV). Serum samples were obtained from SLE patients recruited for the study. Only SLE patients’ sera with dengue serology negative IgG and IgM were used. Neutralization assays against the three major DENV serotypes were performed. Among the dengue serology negative SLE patients’ sera, 60%, 61% and 52% of the sera at 1/320 dilution showed more than 50% inhibition against dengue type-1 virus (DENV-1), DENV-2 and DENV-3, respectively. The neutralizing capacity of the sera was significant against DENV-1 ($P<0.001$) and DENV-3 ($P<0.01$) in comparison to that against DENV-2 ($P<0.05$). The neutralization against the DENV correlated with dengue-specific IgG titer detected below the dengue-positive cut-off value in the serum. Depletion of total IgG from the SLE patients’ sera resulted in significant decrease, up to 80%, in the DENV inhibition ability, suggesting the important role of IgG. Some of the SLE-sera, however, retained the ability to neutralize DENV, even with IgG titers <0.1 O.D. absorbance. Findings from this study suggested that SLE patients’ sera consisted of IgG and possibly other type of antibodies that could cross-neutralized DENV. This could explain the rare reporting of severe dengue among SLE patients. Further studies, however, would be needed to further substantiate this finding and to elucidate the specific neutralizing epitopes recognized by the SLE patients’ sera.

**Keywords**: autoantibodies, autoimmunity, dengue, serology, systemic lupus erythematosus
INTRODUCTION

Dengue virus (DENV) comprises of four closely-related serotypes; dengue type-1 virus (DENV-1), DENV-2, DENV-3 and DENV-4. The virus is transmitted mainly by Aedes sp. mosquitoes. Infection with the virus can result in dengue fever, typically characterized by headache, rash, arthralgia, myalgia and thrombocytopenia, which usually subside without any significant complications. However, in a few instances, severe dengue with evidences of intravascular leakages, bleeding and organ impairments may occur. Manifestations of severe dengue displayed a number of autoimmune disease features, including cross-reaction between the virus-induced antibodies and that of the host cells such as the endothelial cells and platelets that could contribute to induction of plasma leakage and platelet damage (1-3). A number of earlier studies have focused on the immune responses as among the mechanisms contributing to severe dengue. These include the cross-protective ability or non-neutralizing effects of pre-existing antibodies from previous DENV infection against the subsequent infection with the same or different DENV serotypes (4-6).

It has been suggested that homotypic immunity gained from exposure to specific DENV serotype can last a lifetime, whereas heterotypic immunity accorded by the cross-reactive antibodies against other DENV serotype can only last for a limited time (approximately 3 to 6 months) (7). Other findings suggest that cross-reactive heterotypic antibodies from a preceding DENV infection may play important roles in severe dengue (8-9). This is consistent with the finding that most of those who succumbed to severe dengue had previously been exposed to dengue (10). In contrary, cross-protective antibodies between two different viruses have also been reported involving monoclonal antibodies isolated from dengue patients conferring protection against Zika virus (11).
Acquisition of protective antibodies against a particular type of virus is not limited to only previous infection by the same virus or closely related virus, but may also come from cross-viral infections or cross-diseases (11-16). Nevertheless, studies on the potential of cross-protective antibodies against DENV acquired without known previous infection are still lacking.

Autoimmune disease is a disorder caused by irregular immune responses resulting in damages to the host. There are over 80 different autoimmune diseases and among the more prominent one include psoriasis, rheumatoid arthritis, multiple sclerosis, Graves’ disease and systemic lupus erythematosus (SLE). Mechanisms of autoimmune diseases generally have been described as the failure of self-tolerance and existence of auto-antibodies and self-reactive T cells with or without infection or initial host cells damages. SLE is one of the autoimmune diseases that causes an abnormal immune response, potentially affecting the brain, blood and kidney of the patient (17). Similar to many other autoimmune diseases, SLE are typically identified by detection of autoantibodies such as the antinuclear antibodies (ANA) (18). Earlier studies have shown that autoreactive antibodies in SLE patients neutralized human immunodeficiency virus (HIV) (13-14), and this highlights the potential role of cross-reacting autoantibodies in protection against HIV, which contributed in the low frequency of HIV among SLE patients (14).

Reports of severe dengue occurring among patients with autoimmune diseases including SLE have been scarce if any was reported in the literature. Though there were reports of autoimmune diseases occurring among dengue patients (19-20), the inverse, where patients with autoimmune disease, such as SLE, develops severe dengue has not been reported in the literature raising the possibility that patients with autoimmune disease such as SLE are protected against DENV infection and/or severe dengue
comparable to that observed for HIV infection. In this study, we examined sera of SLE patients for the ability to limit DENV infection.

MATERIALS AND METHODS

Study approval

The study was approved by the Medical Research & Ethics Committee, Ministry of Health Malaysia (reference number: NMRR-12-1412-13606) and University Malaya Medical Centre (UMMC) Medical Ethics Committee (MECID No: 2015111832). All samples were taken with informed written consent from the volunteers.

Patient Samples

A total of 132 blood samples of SLE patients were obtained. Volunteers were recruited and selected based on the 1982 American College Rheumatology (ACR) Revised criteria for Classification of Systemic Lupus Erythematosus (21). Blood samples of healthy donors and dengue-positive with no clinically confirmed SLE were obtained from the Tropical Infectious Disease Research & Education Centre (TIDREC) biological samples repository. All blood samples were centrifuged (Heraeus Labofuge 400; ThermoScientific, Waltham, MA) at 2000 ×g for 10 minutes. Serum layer was collected and stored at -80°C until needed. Serum samples were screened for the presence of dengue antibodies using the dengue IgG and IgM capture ELISA (E-protein; Standard Diagnostics, Inc., Gyeonggi-do, Korea). Among the 132 SLE samples, 82 were negative for dengue (IgM/IgG) by ELISA. These samples were used for further studies. Dengue IgG/IgM negative sera of healthy volunteers (n=24) and those positive for dengue IgG/IgM (n=14) were used as controls (Table 1). Patients’ demographic showed no differences in age between SLE and healthy donors, while the mean of the dengue-positive patients’ age was slightly higher. The deficiency of dengue-positive serum from
younger patients was due to the constraint of serum samples collection. SLE samples obtained from the female patients outweigh the male patients by a ratio of 10:1, respectively (Table 1), a ratio expected of SLE population (female: male; 9:1) (22). The numbers of samples used, however, were not equal to each other due to sample collection limitation. Detection of Japanese Encephalitis virus (JEV) antibodies in the sera was performed using anti-JEV ELISA (IgG) (glycoprotein E; Euroimmune AG, Lubeck, Germany). The detection of anti-JEV ELISA (IgM) was not performed due to the sample restriction. Depletion of IgG from sera was performed using ProteoExtract Albumin/IgG Removal Kit (Calbiochem, San Diego, CA) strictly following the manufacturer’s protocol.

Cells and viruses

African green monkey kidney epithelial (Vero) cells were used in this study. Cells were maintained in the Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Bovogen, Australia). Cells were maintained in an incubator (Sanyo, Japan) at 37°C supplemented with 5% CO₂ and subpassaged regularly once they become confluent. DENV-1 (strain 311012), DENV-2 (strain 99133) DENV-3 (strain 3116828) and JEV used in this study were obtained from the WHO Collaborating Centre at University of Malaya (WHOCC, UM). Virus stock was prepared by infecting the Vero cell monolayers. Infected cells were incubated for 7 days at 37°C and 5% CO₂. The supernatants containing the viruses were collected and syringe-filtered prior to keeping at -80°C

Virus titration

Virus stock titers were determined using the focus-forming assay as previously described (23). Vero cells were seeded overnight in the 24-wells plate (Corning, NY) at a cell
density of $7.5 \times 10^4$ per well in DMEM supplemented with 10% heat-inactivated FBS at 37°C with 5% CO₂. Virus stock was diluted at 10-fold dilution and added to the Vero cells. Cells were infected with mild rocking of the plates at room temperature for 1 hour 30 minutes. The infecting inoculum was removed and overlay medium containing DMEM supplemented with 2% FBS and 1.5% carboxymethylcellulose (CMC) (Sigma Aldrich Chemical) was added into each well. The cells were incubated at 37°C with 5% CO₂ for 4 days (DENV-1 and DENV-3) or 3 days (DENV-2).

**Foci staining**

After the incubation, the overlay medium was removed and the cells were fixed using 4% paraformaldehyde (Sigma-Aldrich Chemical, St. Louis, MO) diluted in phosphate buffered saline (PBS; Sigma-Aldrich Chemical, St. Louis, MO) for 20 minutes. The cells were washed thrice with PBS and then 1% Igepal CA-630 detergent (Sigma-Aldrich Chemical, St. Louis, MO) was added for 15 minutes to increase the cell permeability. Cell monolayers were blocked using 3% skimmed milk (Sunlac, Malaysia) for 2 hours at room temperature. Human serum (dengue-positive) was used as the primary antibody in this study and added at 1:500 dilution to the cells. The cells were incubated for 1 hour at 37°C and subsequently washed using PBS. Peroxidase-conjugated goat anti-human-IgG serum (Sigma-Aldrich Chemical, St. Louis, MO) was used as the secondary antibody at 1:250 dilution and incubated at 37°C for 1 hour. Calorimetric detection of the foci was accomplished using metal enhanced 3, 3'-diaminobenzidine (DAB) substrate kit (ThermoScientific, Waltham, MA). The number of foci-formed suggested the titers of infectious virus and recorded as foci-forming unit per millilitre (FFU/ml).

**Foci Reduction Neutralization Test (FRNT)**
Serum neutralization was assayed using the foci reduction neutralization test (FRNT) as previously described (24). Vero cells were seeded into 24-wells plate at $7.5 \times 10^4$ cells per well and kept overnight at 37°C in a 5% CO₂. Serum samples were heat-inactivated at 56°C for 30 minutes to inactivate the complement and subsequently serially diluted (4-fold; 1:20 to 1:1280). Virus stock was diluted to 100 FFU per well and added to the diluted serum. Mixtures of serum and viruses were incubated at 37°C for 1 hour and then introduced to the seeded cells. Virus adsorption was performed by gentle rocking at room temperature and the overlay medium was then added to each well. Cells were incubated at 37°C in 5% CO₂ for 4 days (DENV-1 and DENV-3) or 3 days (DENV-2). Foci staining was performed as described earlier. The virus foci reduction percentage was calculated by comparing the virus that had been exposed to the serum of patients and healthy donors with the virus without exposure to the serum. The serum dilution that reduced the number of foci to 50% was defined as FRNT50.

**Statistical Analysis**

Virus reduction percentage of SLE, dengue-positive and healthy donors’ sera was analyzed using Kruskal-Wallis test alongside Dunn’s test with 95% confidence intervals. Percentage of virus reduction difference between groups was considered significant when $P < 0.05$. The association between DENV neutralization percentage and IgG ELISA titer was determined by using the Spearman test. A significant association of DENV neutralization percentage and IgG ELISA titer was considered significant when $P < 0.05$. Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA).
RESULTS

DENV neutralization by SLE patients’ sera

In order to determine the neutralization capability of SLE patient’s sera against DENV, FRNT assay was performed (24). Eighty two (n=82) of the dengue IgM and IgG negative SLE patients’ sera were used. Sera were diluted at various dilutions (A; 1/20, B; 1/80, C; 1/320, D; 1/1280) and evaluated for neutralization against DENV-1, DENV-2, and DENV-3. At the serum dilution of 1/20, 97% of the SLE-sera, 100% of the dengue-positive sera and 54% of healthy donors with dengue IgM and IgG negative sera showed more than 50% reduction against DENV-1 (Fig. 1A). At higher serum dilution (1/80), both SLE patients’ sera and dengue-positive sera showed 81% and 100% FRNT50 neutralization. Only 33% of the healthy donors’ sera exhibited more than 50% reduction against the DENV-1 (Fig. 1B). The similar pattern was observed at the dilution of 1/320 where 60% of SLE patients’ sera and 93% of dengue-positive sera showed FRNT50 neutralization against DENV-1, while only 13% of healthy donors’ sera with FRNT50 neutralization capacity (Fig. 1C). At the highest serum dilution (1/1280) used, FRNT50 reduction percentages were also reduced in all groups of sera (Fig. 1D). At the lowest serum dilution (1/20), 91% of SLE patients’ sera, 100% of dengue-positive sera and 74% of healthy donors’ sera with FRNT50 against DENV-2 were observed (Fig. 2A). The significant differences in virus inhibition percentages among the sera groups were more distinct at the dilution of 1/80, where the FRNT50 neutralization against DENV-2 of SLE patients’ sera and dengue-positive sera were 74% and 100%, respectively, whereas the healthy donors’ sera were only 47% (Fig. 2B). At higher serum dilution (1/320), 61% of SLE patients’ sera and 100% dengue-positive sera displayed more than 50% reduction against DENV-2, while only 32% neutralization was observed from the sera of healthy
At 1/1280 serum dilution, 50% of dengue-positive sera showed more than 50% reduction against DENV-2 and both SLE patients’ sera and dengue-negative sera exhibited 26% and 11% of FRNT50 neutralizing sera, respectively (Fig. 2D). About 91% of the SLE patients’ sera, 100% of dengue-positive sera and 81% of healthy donors’ sera showed more than 50% reduction against DENV-3 at a serum dilution of 1/20 (Fig. 3A). Both SLE patients’ sera and dengue-positive sera maintained high FRNT50 sera at a serum dilution of 1/80, 74% and 100%, respectively, whereas only 27% of sera of healthy donors showed more than 50% DENV-3 inhibition (Fig. 3B). Comparably, at a serum dilution of 1/320, 52% of SLE patients’ sera and 75% of dengue-positive sera presented more than 50% DENV-3 reduction while none of the healthy donors’ sera showed FRNT50 (Fig. 3C). At the highest serum dilution (1/1280), all sera groups showed less than 50% sera with FRNT50 neutralization against DENV-3 (Fig. 3D).

The neutralization percentages of all sera groups decreased with higher serum dilution, reflecting the concentration-dependent of DENV-1, DENV-2 and DENV-3 virus inhibition. The neutralization effects of SLE patients’ sera against DENV-1 ($P < 0.001$), DENV-2 ($P < 0.01$) and DENV-3 ($P < 0.05$) were significantly higher in comparison to healthy donors’ sera and displayed almost similar capacity to that of dengue-positive sera at serum dilution of 1/320 (Fig. 1, Fig. 2 and Fig. 3). Nevertheless, the neutralization effects of SLE patients’ sera showed no significant differences at serum dilution of 1/20 and 1/80 against DENV-2 (Fig. 2A, 2B) and serum dilution of 1/20 against DENV-3 (Fig. 3A) in comparison to healthy donors’ sera. The neutralization effects of SLE-sera against DENV-3 (**, $P < 0.01$, Fig. 3C, 3D) were better than that observed against DENV-2 (*, $P < 0.05$, Fig. 2C, 2D), though not as high as that against DENV-1 (***, $P < 0.001$, Fig. 1C, 1D). Moreover, virus reduction by healthy donors’ sera was higher against DENV-2
in comparison to DENV-1 and DENV-3, which contributed to the reduced neutralizing effects of SLE patients’ sera against DENV-2 (Fig. 2B, 2C). Results obtained suggested that SLE patients’ sera neutralized DENV-1, DENV-2 and DENV-3, but with varying capacity against the virus serotype with DENV-1 being the most susceptible and DENV-2 being the least. In addition to sample limitation, neutralization assay against DENV-4 was not performed due to the low prevalence of DENV-4 infection in Malaysia in over three decades (25).

**IgG plays a role in the neutralization of DENV by SLE patients’ sera**

We examined the potential role of IgG presence in the SLE-sera. IgG was depleted using ProteoExtract Albumin/IgG removal kit. Due to the limited amount of sera available, only 6 SLE patients’ sera were randomly chosen and used in this study. From the 6 SLE patients’ sera, the amount of dengue-specific IgG post-depletion was determined prior to the neutralization assay against DENV-1 and DENV-2. IgG-depleted serum of SLE patient 1 at the dilution of 1/320 caused 34% and 46% decreased, whereas SLE patient 2 showed 30% and 53% decreased in the DENV-1 and DENV-2 reduction percentage, respectively (Fig. 4A, 4B). SLE patient 3 displayed 24% and 81% declined while SLE patient 4 showed 71% and 86% decreased in the reduction percentage of DENV-1 and DENV-2, respectively, at the serum dilution of 1/320 after depletion of IgG (Fig. 4C, 4D). The decreased in the reduction percentage of the viruses was also observed in SLE patient 5 (69% [DENV-1] & 67% [DENV-2]) and SLE patient 6 (31% [DENV-1] & 53% [DENV-2]) at the dilution of 1/320 (Fig. 4E, 4F). The total reduction of dengue-specific IgG antibody in the sera was about 50% to 60% (Fig. 4G). These results suggest that IgG in the sera of SLE patients is important for the neutralization of DENV.
Correlation between DENV-neutralizing capacity of naive SLE patients’ sera and cross-reacting IgG and IgM

Given the involvement of IgG and IgM in the neutralization of DENV (26), we investigated the relationship between neutralization percentage of SLE patients’ sera against DENV and the level of dengue IgG and IgM titer. As mentioned earlier, all of the SLE patients’ sera used in this study were defined as negative for dengue IgG (below ELISA cut-off value <0.390 O.D. absorbance) and IgM (below ELISA cut-off value <0.345 O.D. absorbance) by ELISA. Though determined as negative using the cut-off value, these samples showed varying O.D. ranges from 0.00 to 0.345 for IgM and 0.389 for IgG titer. The O.D. absorbance results of each sample were compared against its DENV neutralization percentage. Higher dengue IgG and IgM titer, as expected, caused higher neutralization percentage against DENV-1, signifying a positive correlation between DENV-1 reduction percentage and IgG and IgM ELISA titer (Fig. 5A, 6A). However, DENV-2 reduction percentage showed insignificant correlation with IgG and IgM ELISA titer (Fig. 5B, 6B). Association of DENV-3 reduction percentage and IgG was only significant at the 1/80 serum dilution but insignificant at 1/320 serum dilution (Fig. 5C) whereas positive correlation between IgM and neutralization capacity against DENV-3 can be observed at both dilutions (Fig. 6C). At the serum dilution of 1/320, 22% and 30% of the SLE-sera showed neutralization capacity against DENV-1 (>50% virus reduction), even with low to undetectable dengue IgG and IgM ELISA titer (<0.1; O.D. absorbance), respectively (Fig. 5A, 6A; black box). Moreover, about 34% and 33% of SLE-sera with low dengue IgG and IgM titer (<0.1; O.D. absorbance), respectively, showed more than 50% DENV reduction against DENV-2 (Fig. 5B, 6B; black box). About 28% and 21% of SLE-sera with low dengue IgG and IgM titer (<0.1; O.D.
absorbance), respectively, showed more than 50% DENV reduction against DENV-3 (Fig. 5C, 6C; black box). These findings suggested that the neutralizing capacity of the SLE patients’ sera against DENV correlated with the presence of dengue virus specific IgG and IgM. However, the inherent neutralizing ability of sera with low dengue-specific IgG and IgM ELISA titer could possibly cause the statistically insignificant correlation demonstrated between dengue-specific IgG and IgM titer, and the neutralizing capacity of SLE-sera against DENV-2 and DENV-3 (Fig. 5B, 5C and 6B). Nevertheless, the neutralization effects of the low dengue-specific IgG ELISA titer sera were not observed among the dengue-positive and healthy donors’ sera, which displayed significant positive correlation between DENV-1, DENV-2 and DENV-3 reduction percentage and the dengue IgG ELISA titer (Supplemental Fig. 1A, 1B and 1C). Hence, these suggest the possibility that the neutralizing effects of SLE patients’ sera may not solely caused by the IgG or IgM.

**SLE patient’s sera do not neutralize JEV**

We examined if the SLE-serum neutralization against DENV was specific by performing similar neutralization assays against a closely related *Flavivirus* known endemic in Malaysia and the regions (27), the Japanese Encephalitis virus (JEV). SLE patients’ sera, dengue-positive patients’ sera and healthy donor’s sera were diluted to 1/20, 1/80, 1/320 and 1/1280 dilution and FRNT were then performed. Dengue-positive patients’ sera were also tested to examine the potential of cross-neutralization effects against JEV. At the serum dilution of 1/20, 75% of SLE patients’ sera, 100% of dengue-positive sera and 88% of healthy donors’ sera showed 50% or more inhibition against JEV (Fig. 7A). The percentages of SLE patients’ sera and dengue-negative sera with the capacity to neutralize 50% of JEV were 22% and 25%, respectively, while 80% of dengue-positive showed
FRTN50 at the serum dilution of 1/80 (Fig. 7B). At the serum dilution of 1/320 and 1/1280, all groups of sera displayed less than 50% of FRNT50 sera against JEV (Fig. 7C and 7D). The neutralization effects of SLE patients’ sera showed no significant differences in comparison to the healthy donor’s sera at all dilutions, suggesting the inability of SLE patients’ sera to discriminatively neutralize JEV. Dengue-positive patients’ sera also showed no significant neutralization against JEV when compared to dengue-negative and SLE patients’ sera. These suggested that there were no significant cross-neutralization of dengue positive and SLE patients’ sera against JEV. Anti-JEV ELISA (IgG) assay, however, demonstrated that more than half of the SLE patients’ sera were positive (above ELISA cut-off value: >1.1 O.D. absorbance) (Supplemental Fig. 2A), hence, suggesting that JEV IgG ELISA titer displayed no correlation to the neutralization capacity against JEV. High titer of JEV IgG in SLE patients’ and dengue-positive patients’ sera showed low percentages of neutralization against JEV, especially at the dilution of 1/320 (Supplemental Fig. 2A and 2B). Taken together, these data revealed that the high cross-reactivity of SLE patients’ sera against JEV was only apparent from the IgG ELISA titer but not the neutralization capacity against JEV. JEV IgM titer in the SLE patient serum was not determined due to sample limitation.
DISCUSSION

Results obtained from the study suggest that SLE patients’ sera have the capacity to effectively neutralize DENV. The neutralization effects of the SLE patients’ sera can be seen up until the dilution of 1/1280, suggesting high inhibitory titer against DENV. Some of the healthy donors’ sera also showed high neutralization against DENV, particularly at the lowest serum dilution (1/20), where the neutralization effect was probably due to the non-specific reaction, influenced by the high concentration of the serum (28). However, there were a few healthy donor’s sera that showed high neutralization against DENV, even at higher serum dilutions (1/80 and 1/320). Although regarded as negative for dengue serology using dengue IgG and IgM ELISA assays, these healthy donors probably had encountered other closely-related virus infection, which led to the presence of non-specific recognition towards DENV. On the other hand, we could not ascertain if the healthy volunteers had an underlying undiagnosed autoimmune disease.

During the initial screening of all the SLE patients, 40% among the volunteers were positive for the presence of dengue virus IgG (data not shown). This relatively high percentage of positive dengue serology among the SLE patients, however, was not in concordance with the very rare or possibly none reported cases in the literature, of severe dengue among SLE patients. In the present study cohort, there was only one reported hospitalized case of an SLE patient diagnosed with dengue, but the patient was discharged as an outpatient (29), suggesting that she had only mild symptoms. In the literature, most cases involving dengue and autoimmune disease were reported as a progression to the development of autoimmunity in patients from the initial DENV infection (30-32).

Our findings in this study, however, was not surprising considering the almost similar observation made in a study involving 400 HIV patients with SLE, where only
5% of them were simultaneously displaying autoimmunity and HIV infection (33-35). Further studies showed that SLE patients’ sera can effectively neutralize HIV virus (16-36-39). HIV patients with autoimmune disease were also found to be more readily to produce autoantibodies such as broadly neutralizing antibodies (bNAbs), a highly potent antibody against HIV, than those without autoimmune disease (36). Moreover, patients with defective immune tolerance may have protection against HIV due to the production of antibody-dependent cellular cytotoxicity (ADCC), a response initiated early after HIV infection (37). It was found that the level of ADCC was inversely correlated with the onset of acute HIV (38) and shown to protect HIV transmission from mother to the infant (39). In addition, anti-phospholipid monoclonal antibody isolated from an SLE patient inhibited HIV infection by releasing soluble chemokines that block the entry of the virus into the host (16). Another autoantibody called anti-cardiolipin that is prevalent among SLE patients (40) showed an association with the neutralization of HIV-1 by recognizing the envelope glycoprotein of the virus (41). Anti-protein disulphide isomerase (anti-PDI) and anti-heat shock protein 60 (anti-HSP60) also correlate with anti-DENV NS1 IgM titer, suggesting the sequence homology between the proteins and the virus (42). Both IgM and IgG antibodies, either naturally produced or virus-specific, may possess a moderate to high binding avidity to the host surface protein, where virus-binding activity and virus entry are probably inhibited (43-44). Binding of the antibodies or autoantibodies to the host cell surface protein such as Toll-like receptors may also activate dendritic cells to induce type-1 interferon production and other immune responses against viruses (43). Taking these findings together, it is possible that the rare reporting of dengue, especially severe dengue among the SLE patients, could reflect the same protective mechanisms, which involve the neutralization of the virus by the autoantibodies.
In the present study, our data revealed that the neutralizing capacity of the SLE-sera correlated with the dengue IgG and IgM levels. The importance of IgG was highlighted from the study using the IgG-depleted sera. Depletion of IgG resulted in significantly decreased ability of the sera to neutralize DENV. The finding is in agreement with the earlier studies with other viruses, where IgG is crucial in the protection against simian-human immunodeficiency virus (SHIV) (45), measles and human cytomegalovirus (46). After depletion of IgG from SLE-sera, the O.D absorbance of dengue IgG ELISA results of most of the sera were lower than 0.1 (Fig. 4G). Even so, some of the SLE patients’ samples with low dengue IgG and IgM ELISA titer, still showed neutralization effects against DENV. This suggests that the neutralization of DENV by SLE-sera took place even when the dengue-specific IgG and IgM was below detectable levels. It was also interesting to note that the reduction of neutralization capacity by the IgG-depleted sera, however, was not in concordance with the high neutralization capability of some of the undepleted sera with similarly low dengue IgG ELISA titer (Fig. 4G; SLE patient 4). The anomaly could be due to the reduction of other unknown proteins or low abundance antibody in the process of IgG depletion (47), which probably play an important role in the neutralization activity, in addition to IgG. The possibilities of the involvement of other antibodies could not be ruled out. IgE, which is known to be high in SLE patients (48-49), were not examined in this study. IgE antibody levels, however, was previously reported to be high in the convalescent phase sera of dengue fever patients (50), raising the possibility that the antibody could be involved. In HIV infection studies, inhibition of HIV replication was enhanced in the cell culture added with serum containing anti-HIV-IgE, supporting the role of IgE in antiviral responses (51). Furthermore, HIV patient with hyper-IgE serum showed that IgE could
confer protection against HIV replication (52). The importance of IgE was also observed in Parvovirus B19 infection (53). While it is possible that the presence of IgE against DENV in the sera of SLE patients could have contributed to the virus neutralization effects, especially in sera with low IgG titer, the study was not performed here, due to limitations of serum sample availability.

Results from the study, nonetheless, suggest high specificities of SLE patient’s sera in neutralizing DENV, as JEV, a virus of the same genus was not specifically neutralized by the SLE patients’ sera. Our data showed no significant neutralization ability of SLE patients’ sera against JEV, despite being positive for anti-JEV by ELISA (IgG) assay. This finding is comparable to that observed in the high cross-reactivity between dengue- and JEV-positive sera in the hemagglutination inhibition (HI) but low neutralization capacity against each other, owing to the fact that DENV and JEV possess less antigenic similarity (54). These results suggest that the SLE patient’s sera might recognize antibody recognition sites on DENV but not JEV.

In summary, sera from dengue serology negative SLE patients were found to have the capacity to neutralize DENV. The neutralization is dependent for the most part on the presence of dengue-specific IgG antibodies and to a lesser extent potentially other yet to be identified antibody isotypes or cross-reacting antibodies. These observations may pave a way to further understand the broader implications of autoimmune diseases and viral infections especially in contact of the high endemicity of dengue and prevalence of SLE in the Southeast Asian regions.
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DISCLOSURE

The authors declared no conflict of interest in the publication of this manuscript.
REFERENCES


FIGURE LEGENDS

**Fig. 1.** SLE-serum neutralizing activity against DENV-1. Heat-inactivated dengue-positive (n=15), dengue-negative (n=24) and SLE patients’ (n=67) sera were diluted into 1/20 (A), 1/80 (B), 1/320 (C), 1/1280 (D) and incubated with DENV-1 for 1 h. The incubated solution was then added to Vero cells and the virus titers were determined using focus forming assay. Neutralization potency was verified with 50% virus inhibition. Values represent the mean ± SEM percentage of DENV-1 reduction compared between dengue-positive sera, dengue-negative sera and SLE patients’ sera. Significant differences between the groups are shown as ns: not significant, *P <0.05, **P <0.01, ***P <0.001 by Kruskal-Wallis test with Dunn’s multiple comparison post-test analysis. Figure was created using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA).

**Fig. 2.** SLE-serum neutralizing activity against DENV-2. Heat-inactivated dengue-positive (n=14), dengue-negative (n=19) and SLE patients’ (n=70) sera were diluted into 1/20 (A), 1/80 (B), 1/320 (C), 1/1280 (D) and incubated with DENV-2 virus for 1 h. The incubated solution was then added to Vero cells and the virus titers were determined using focus forming assay. Values represent the mean ± SEM percentage of DENV-2 reduction compared between dengue-positive sera, dengue-negative sera and SLE patients’ sera. Significant differences between the groups are shown as ns: not significant, *P <0.05, **P <0.01, ***P <0.001 by Kruskal-Wallis test with Dunn’s multiple comparison post-test analysis. Figure was created using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA).

**Fig. 3** SLE-serum neutralizing activity against DENV-3. Heat-inactivated dengue-positive (n=12), dengue-negative (n=11) and SLE patients’ (n=82) sera were diluted into
1/20 (A), 1/80 (B), 1/320 (C), 1/1280 (D) and incubated with DENV-3 virus for 1 h. The incubated solution was then added to Vero cells and the virus titers were determined using focus forming assay. Values represent the mean ± SEM percentage of DENV-3 reduction compared between dengue-positive sera, dengue-negative sera and SLE patients’ sera. Significant differences between the groups are shown as ns: not significant, *P <0.05, **P <0.01, ***P <0.001 by Kruskal-Wallis test with Dunn’s multiple comparison post-test analysis. Figure was created using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA).

**Fig. 4.** Neutralizing capacity of SLE-patients’ sera following IgG depletion. Total and IgG-depleted sera of SLE patient 1 (A), 2 (B), 3 (C), 4 (D), 5 (E), and 6 (F) were heat-inactivated and diluted into 1/80, 1/320, and 1/1280. Next, sera were incubated with DENV-1 and DENV-2 virus for 1 h. The incubated solution was then added to Vero cells and the virus titers were determined using focus forming assay. Dengue IgG titer depletion was confirmed using dengue IgG capturing ELISA (G). Figure was created using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA).

**Fig. 5.** Association between dengue neutralization percentage and dengue IgG titer among SLE patients. SLE patients’ sera diluted into 1/80 and 1/320 were incubated with DENV-1 (SLE n=67) (A), DENV-2 (SLE n=70) (B) and DENV-3 (SLE n=82) (C) for 1 h. The incubated solution was then added to Vero cells and the virus titers were determined using focus forming assay. Dengue IgG in the serum was determined using dengue IgG capturing ELISA. Association between DENV neutralization percentage and dengue IgG ELISA titer (O.D. of serum samples) of SLE patients’ sera was determined using Spearman test and correlations were indicated by Spearman ρ value and the P value. Figure was created using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA).
Fig. 6. Association between dengue neutralization percentage and dengue IgM titer among SLE patients. SLE patients’ sera diluted into 1/80 and 1/320 were incubated with DENV-1 (SLE n=67) (A), DENV-2 (SLE n=70) (B) and DENV-3 (SLE n=82) (C) for 1 h. The incubated solution was then added to Vero cells and the virus titers were determined using focus forming assay. Dengue IgM in the serum was determined using dengue IgM capturing ELISA. Association between DENV neutralization percentage and dengue IgG ELISA titer (O.D. of serum samples) of SLE patients’ sera was determined using Spearman test and correlations were indicated by Spearman ρ value and the P value. Figure was created using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA).

Fig. 7. SLE-serum neutralization activity against JEV. Heat-inactivated dengue-positive (n=5), dengue-negative (n=8) and SLE patients’ (n=36) sera were diluted into 1/20 (A), 1/80 (B), 1/320 (C), 1/1280 (D) and incubated with JEV for 1 h. The incubated solution was then added to Vero cells and the virus titers were determined using focus forming assay. Values represent the mean ± SEM percentage of JEV reduction compared between dengue-positive sera, dengue-negative sera and SLE patients’ sera. Significant differences between the groups are shown as ns: not significant, *P <0.05, **P <0.01, ***P <0.001 by Kruskal-Wallis test with Dunn’s multiple comparison post-test analysis. Figure was created using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA).
SUPPORTING INFORMATION

Supplemental Fig. 1. Relationship between dengue neutralization percentage and dengue IgG titer among dengue-positive and healthy donor’s sera.

Supplemental Fig. 2. Association between neutralization percentage against JEV and JEV-specific IgG ELISA titer.
### ABBREVIATION

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACR</td>
<td>American College Rheumatology</td>
</tr>
<tr>
<td>ADCC</td>
<td>antibody-dependent cellular cytotoxicity</td>
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<tr>
<td>ANA</td>
<td>antinuclear antibodies</td>
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<tr>
<td>anti-HSP60</td>
<td>anti-heat shock protein 60</td>
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<tr>
<td>anti-PDI</td>
<td>anti-protein disulphide isomerase</td>
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<tr>
<td>bNAbS</td>
<td>broadly neutralizing antibodies</td>
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<tr>
<td>CMC</td>
<td>carboxymethylcellulose</td>
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<tr>
<td>DAB</td>
<td>diaminobenzidine</td>
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<tr>
<td>DENV</td>
<td>dengue virus</td>
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<tr>
<td>DMEM</td>
<td>Dulbecco’s modified Eagle medium</td>
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<tr>
<td>FBS</td>
<td>fetal bovine serum</td>
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<tr>
<td>FFU</td>
<td>foci-forming unit</td>
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<td>FRNT</td>
<td>foci reduction neutralization test</td>
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<tr>
<td>HI</td>
<td>haemagglutination inhibition</td>
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<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
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<td>JEV</td>
<td>Japanese Encephalitis virus</td>
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<td>PBS</td>
<td>phosphate buffered saline</td>
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<td>SHIV</td>
<td>simian-human immunodeficiency virus</td>
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<tr>
<td>SLE</td>
<td>systemic lupus erythematosus</td>
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<td>TIDREC</td>
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<tr>
<td>UMMC</td>
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<tr>
<td>Vero</td>
<td>African green monkey kidney epithelial (Vero)</td>
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TABLES

Table 1 Demographics of patients and healthy sample donors used for the study

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Number of subjects, age and gender of the SLE patients, dengue-positive patients and healthy (autoimmune disease negative and dengue-negative) donors.
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6
Figure 7