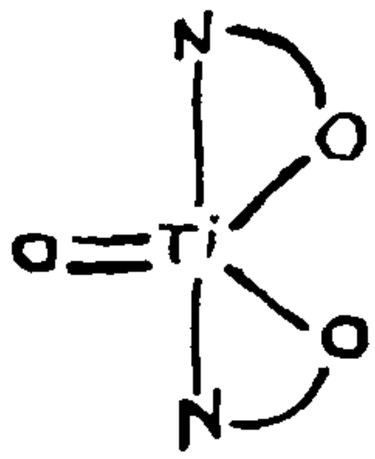
trigonal bi-pyramidal structure with intermolecular hydrogen bonding is proposed for the present series.



ACKNOWLEDGEMENT

One of the authors (M. D. P.) acknowledges with thanks C.S.I.R., for the award of the Junior Research Fellowship.

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PREVALENCE OF SOLUBLE COMPLEMENT FIXING (SCF) ANTIBODY TO JAPANESE ENCEPHALITIS (JE) VIRUS IN CASES OF JE VIRUS INFECTION

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ABSTRACT

Serial samples of human sera belonging to confirmed cases of Japanese encephalitis (JE) virus infection were studied for the presence of antibody to soluble complement fixing (SCF) antigen of JE virus.

All the convalescent phase sera (39) out of a total of 44 sera tested, were positive for JE SCF antibodies whereas all the acute phase sera (5) were negative for these antibodies. There was complete correlation between results of complement fixation (CF) and Agarose gel diffusion (AGD) tests with JE SCF antigens. Use of JE SCF antigen as a useful reagent for specific serodiagnosis of JE virus infection bv CF and AGD tests is suggested.

FALKLER *et al.* (1973) detected SCF antibodies exclusively in sera of secondary dengue cases in convalescent phase of the infection. They were unable to detect SCF antibodies in acute and primary convalescent phase sera of patients with dengue infection.

Presence of SCF antibodies in human sera in Japanese encephalitis (JE) virus infection has been demonstrated recently (Rai *et al.*, 1975) by complement fixation (CF) and Agarose gel double diffusion (AGD) tests. However, only a limited number, mostly secondary type convalescent phase sera were found to be positive for JE SCF antibodies.

In view of the absence of correlation between HI, CF and/or neutralizing antibodies and SCF antibodies, more sera from confirmed cases of JE virus infection were tested.

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MATERIAL AND METHODS

Viruses.—JE Virus (VRC Strain P 20778) plaque purified in Vero cells was used for preparation of JE SCF antigen. SCF antigens of West Nile (Strain G 22886), DEN-1 (Hawaii), DEN-2 (Strain P 23085 and TR 1751), DEN-3 (Strain 633798), DEN-4 (Strain 642069 and 611319) and Chikungunya (CHIK strain 634029) viruses were also prepared.

SCF Antigens.—SCF antigens were prepared from the infected mouse brain. A 20% suspension of infected brains of the suckling mice in 0·02 M Tris HCl buffer (pH 8·2) was centrifuged at 105,000 × G for 4 hours and upper third of the supernatant fluid was used as SCF antigen. The control SCF antigen was prepared in a similar manner from the brains of normal infant mice inoculated with plain normal saline (0·02 ml each, intracerebrally).

Immune Peritoneal Fluids.—Mouse hyperimmune peritoneal fluids to crude mouse brain suspensions of JE (P 20778), WN (strain G 22886), DEN-1 (Hawaii), DEN-3 (Strain 633798) and Chikungunya (CHIK) viruses and to SCF antigens of these viruses were prepared in adult female mice according to the method described by Brandt *et al.* (1967).

Human Sera.—The sera used in the present study were collected during an outbreak of Japanese encephalitis (JE) virus infection in North Arcot District of Tamil Nadu State, India in 1955 and 1956 and stored at Virus Research Centre, Poona. A total of 44 sera belonging to 14 cases of JE virus infection confirmed clinically and serologically (by HI, CF and/or neutralization tests) as reported earlier (Webb and Pereira, 1956 and Work and Shah, 1956) were available. The sera had been kept all these years at −20° C. These 44 sera were comprised of serial samples, collected during acute and convalescent phases of illness. Sera collected within 7 days after the onset of symptoms were referred to as acute samples whereas those collected after 8 days were considered as convalescent sera.

Complement Fixation Test.—CF tests were done by employing the procedure described by Casals (1967) adapted to micro-techniques in 'U' plates employing 4–8 units of SCF antigen per test volume (0·025 ml).

Agarose Gel Diffusion (AGD) tests.—Double diffusion immunoprecipitation tests were performed on 7 × 2·5 cm microscopic slides employing 1% agarose in 0·05 M borate saline (pH 9·0). Merthiolate (1 : 10,000) or sodium azide (1 : 1000) was used as preservative. About 3·0 ml melted agarose (1%) was sufficient for each slide. Wells were cut in hexagonal pattern (one central well having six peripheral wells) with 4 mm diameter and 8 mm centre to centre distance. SCF antigens, standardized to 256 units, and normal antigen were placed in central wells and the human sera in the peripheral wells.

Results

Clinico-serological classification of the sera tested and results of CF and AGD tests with JE SCF antigen are given in Table I. These results show that all the convalescent phase sera (39) were positive for JE SCF antibodies in CF and AGD tests. The earliest time of detection of JE SCF antibodies was 9 days after the onset of symptoms. Out of these 39 sera positive for JE SCF antibodies 7 sera were of monoreacting type (antibodies against JE virus only) and 32 sera were of cross reacting (secondary) type, showing cross reactions with sucrose acetone extracted mouse brain (SAMB) antigens of other group B arboviruses also. All the 5 acute phase sera were negative for JE SCF antibodies in CF and AGD tests. There was no correlation between antibodies to SAMB antigens and SCF antibodies. Some of the sera which had high HI and CF antibodies to SAMB antigens had low JE SCF antibody titres and *vice versa* too. In one case of late convalescent phase (352 days after onset of symptoms) there were no detectable HI and CF antibodies to SAMB antigens whereas JE SCF antibodies were detected in CF and AGD tests. These 39 sera positive for JE SCF antibodies did not react with SCF antigens of West Nile (WN), dengue (DEN 1-4) and Chikungunya (CHIK) viruses and normal antigen in CF and AGD tests.

Discusson

JE SCF antibodies were detected in all the convalescent phase sera (39), whether nonreacting (1) monoreacting (7) or cross reacting (31) types. On the other hand all the acute phase sera (5), whether nonreacting (2) or monoreacting (2) or cross reacting (1) types were negative for JE SCF antibodies. There was complete correlation between results of CF and AGD tests with JE SCF antigen. All the 39 sera showing JE SCF antibodies in CF tests were also positive in AGD tests with JE SCF antigen, but none of them reacted with SCF antigens of WN, DEN (1-4) and CHIK viruses and normal antigen in CF and AGD tests. These findings have demonstrated a considerable degree of specificity and sensitivity of JE SCF antigen and antibody in CF and AGD tests.

It is too premature to comment on the role of SCF antigen and the significance of SCF antibody in JE virus infection. However, the specificity and sensitivity of JE SCF antigen seen in the serological reactions (CF and AGD tests) suggests that it could be employed as a useful reagent for specific serodiagnosis of JE virus infection.

TABLE I

Antibodies to JE SCF antigen in human sera during an outbreak of JE virus infection in Tamil Nadu (then Madras State) India (1955–1956)

*Type of sera (Clinico-serological classification)	No. of sera tested	*No. positive for antibodies to JE and other group B arbo-viruses (cross-reacting)	*No. positive for antibodies to JE virus only (Mono-reacting)	*No. non-reacting to any of the group B arboviruses antigens (Non-reacting)	No. positive for JE SCF antibodies in CF tests	†No. positive for AGD-test with JE SCF antigen
1. Acute non reacting ..	2	0	0	2	0	0
2. Acute monoreacting ..	2	0	2	0	0	0
3. Acute crossreacting ..	1	1	0	0	0	0
4. Convalescent nonreacting ..	1	0	0	1	1	1
5. Convalescent monoreacting ..	7	0	7	0	7	7
6. Convalescent crossreacting ..	31	31	0	3	31	31
TOTAL ..	†44	32	9	3	39	39

* As determined by SAMB antigens of JW, WN, DEN 1-4 and MVE viruses in HI, CF and/or N tests. Serum samples collected upto 7th day after the onset of symptoms were classified as acute phase sera and those collected after 7 days were considered as convalescent phase sera.

† These 44 sera comprised of serial samples collected during acute and convalescent phases from 14 confirmed cases of JE virus infection.

‡ Sera positive for JE SCF antibodies gave precipitin lines in AGD tests with JE SCF antigen only and not with WN SCF antigen or normal antigen.

ACKNOWLEDGEMENTS

We are thankful to the members of staff in the Division of Immunology, Virus Research Centre, Poona, for their technical assistance in this work.

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