

## Group B Arbovirus Serology: Search for Humoral Specificity

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**OBJECTIVE:** To improve serological specificity in group B arbovirus infections by isolating and titrating serum IgM antibody in individuals with dengue and Japanese encephalitis virus infections.

**BACKGROUND:** A detailed description of this study, together with the IgM antibody serological results on 39 patients with group B arbovirus infections, was included in last year's SMRL Annual Report. Briefly we observed that the 3 standard serological tests, HI, CF, and PRNT, used alone or in combination cannot clearly identify a type-specific group B arbovirus antibody in whole serum of individuals who have been previously infected with group B agents. In an attempt to improve specificity we fractionated convalescent sera obtained from Japanese encephalitis patients by sucrose density gradient centrifugation and found low titered IgM HI antibody directed monospecifically against JEV. The IgG in these sera reacted heterospecifically in high titers with antigens common to dengue 1-4 and JEV and was responsible for the cross reactions noted in standard serological tests.

We report here studies on the specificity and persistence of circulating IgM antibody in Japanese encephalitis patients, IgM patterns in a variety of dengue infections, and the use of IgM analysis to diagnose fevers of unknown origin.

**PROGRESS:** A. Serum IgM Antibody in Japanese Encephalitis Patients, Chiangmai 1970.

The Dept. of Neuropsychiatry, SMRL, has studied the neuropsychiatric recovery of convalescing JE patients. The patients were hospitalized in Chiangmai and adjacent Lamphang Valley in 1970 and were bled for group B arbovirus serology (dengue 1-4 and JEV) during hospitalization and at intervals of weeks to months over a 1 year followup period. No dengue transmission was detected in these valleys in 1970. Access to this serum has provided a unique opportunity to 1) correlate the presence or absence of detectable JEV IgM antibody with the HI and CF serological pattern (primary or secondary infection), 2) determine in a large number of patients whether or not JEV IgM antibody cross-reacts with dengue 1-4 antigens, and 3) study the persistence of circulating IgM antibody following clinical disease.

Table 1 summarizes the serologic criteria used to distinguish primary JEV infections and secondary group B arbovirus infections of unspecified virus type.

A total of 54 encephalitis patients have been studied to date; their whole serum serological patterns and IgM analyses are summarized in Table 2. The number of serum specimens analyzed from each patient ranges from 1 to 6. Of 45 patients showing  $\geq 4$ -fold rising titers, 19 had a primary JEV infection, and 18 of the 19 had JEV specific IgM antibody in 1 or more convalescent sera, thus confirming a first infection with JEV. Fifteen of 26 patients with rising titers and a secondary group B serological pattern had IgM and thus evidence of first infection with JEV. All patients with IgM antibody had recent JEV infections, because IgM was not found in the acute serum or was found in titers significantly lower than in convalescent sera. Presumably these 15 patients had been previously infected by a group B arbovirus other than JEV; this previous heterologous group B infection did not protect them against Japanese encephalitis.

Several possible reasons can be given for the failure to detect IgM in 11 of the 26 patients with serologically confirmed secondary infections. First, the high titered IgG antibody characteristically found in secondary infection sera tends to contaminate the IgM-containing serum sucrose fractions. If IgM antibody titers are lower than contaminating IgG titers in such fractions, they will be 2-mercaptoethanol resistant, and thus falsely appear to contain no IgM. Second, timing of the convalescent serum is important, because the IgM rise may be transient and so be detectable only 10 to 28 days after the onset of illness. For example three IgM negative patients, with serums drawn on days 9, 42, and 45, may fall into this category. Thirdly, some of these 11 negative patients may have been infected with JEV before their recent JE infection and might not produce IgM after a repeat JEV infection. Fourthly, the acute heterospecific rise in whole serum titers may be caused by a group B encephalitis virus infection other than JEV. Finally it is conceivable that some individuals do not synthesize IgM antibody to JEV if they have been previously sensitized to a related group B agent. The latter 3 explanations are considered less likely than the first 2 offered. In addition, JEV-IgM antibody was present in 4 of 9 patients with fixed or falling whole serum titers indicating a recent JEV infection, although it was not possible to diagnosis a recent infection using standard serological tests.

Thirty-seven of 39 patients with IgM antibody listed in Table 2 had titers of IgM antibody reactive only against JEV. Two patients (both with secondary infections) had IgM that cross reacted with D4 (JE-C-69) and D1, D2, and D3 (JE-M-37) (see Table 3). Thus IgM antibody produced by 39 encephalitis patients was monospecific in 95% of cases, and in one of the exceptions with heterospecific IgM antibody (JE-C-69) the high JEV IgM titers and low D4 IgM titers strongly suggested a recent JEV infection. The unusual combination of high titered, cross reactive and persistent IgM antibody found in the other exception, JE-M-37, remains an enigma; her IgM and whole serum patterns are compatible with a recent dengue infection (see next section on dengue infections), however no dengue transmission was documented at the time (1970) and place (rural Chiangmai Valley) where she was infected.

The persistence of circulating IgM antibody was determined in the 19 patients with primary and the 18 with secondary group B serological patterns (Table 2). The results, summarized in Table 4, indicate that IgM may circulate in gradually declining titers for greater than 5 months after primary and secondary infections. The IgM was detected in one patient for at least 14 months. Following sera collected later than 1 month after illness was usually drawn at 2 to 6 month intervals rather than monthly. Many primary infection patients in particular, had IgM antibody present in all serum samples, so that even longer persistence would have undoubtedly been documented with additional or more frequent sampling. The time intervals listed in Table 4 therefore represent the minimum rather than the maximum values for IgM longevity *in vivo*. The question arises whether such long term persistence of IgM is associated with persistence of the antigen, and if so, whether this antigenic persistence is clinically significant. Furthermore, will those patients with prior group B infection as evidenced by a 2° serological response, be partially protected as a consequence of prior sensitization and show fewer neuropsychiatric sequelae (as compared to 1° infections) during hospitalization and thereafter? Finally does a clinical difference exist between those who had detectable IgM antibody and those who do not? All of these questions are being examined now in collaboration with the Department of Neuropsychiatry. There were no discernable age or sex differences between the patient groups with and without IgM antibody; approximately 1/3 were female and 2/3 were male in both groups, and ages ranged from 6 to 35 yrs in IgM negative patients and 3 to 47 years in the IgM positive patients.

#### C. Serum IgM antibody in inapparent JEV infections.

Approximately 31 Chiangmai villagers and urban school children studied in 1970 had serological evidence consistent with recent JE or group B arbovirus infection. Ten persons had primary JEV infections with rising or falling JEV titers; the remaining 21 had secondary infections with rising, falling or fixed titers. 36 serum from these 31 persons were fractionated by S-DGC. Only 1 serum from a Chiangmai City school child contained trace amounts of IgM antibody to dengue 2. The 35 other serum contained no demonstrable

IgM antibody. The inability to detect IgM antibody in these sera may have resulted from the 3 month interval between each bleeding, with decay of IgM antibody in the interim; alternately individuals with inapparent JEV infections may not produce IgM antibody or produce it for much more limited durations than persons with encephalitis.

In order to eliminate the latter possibility, serum obtained from 6 family members of 6 encephalitis patients was fractionated. These family members had experienced an inapparent JEV or group B infection diagnosed by rising titers in serial serums drawn prospectively over 1-3 week intervals. As shown in Table 5, 4 of the 6 subjects produced IgM antibody reactive only with JEV, thus confirming that IgM is produced in inapparent JEV infections.

#### C. Dengue infections.

The IgM results on 2 patients with dengue hemorrhagic fever (DHF) (2° infections) and 6 patients with dengue fever (1° infection) were reported in last year's Annual Report. Briefly, no IgM antibody was found in the 2 patients with DHF and in one with 1° dengue fever. Five dengue fever patients did have IgM antibody in their convalescent serum, but in 2 of these individuals the IgM cross reacted with either JEV or another dengue serotype. Four more patients admitted to Bangkok hospitals in 1971 were studied and are reported below. Three had clinically confirmed DHF (CH-404k, CH408k, CH-454m; Table 6) and 1 (RA-005k) had unconfirmed DHF (hospital record not available). These 4 children were 4 to 6 months old and all had serological patterns consistent with 1°, rather than 2° dengue infections. Dengue 2 virus was isolated from 1 patient who also developed severe dengue shock syndrome. Low serum complement levels were found in the 2 patients tested for C' (CH-404k, CH-408k,). The IgM patterns shown in Table 6, reveal that all 4 developed IgM antibody to dengue, but unlike 95% of JE patients, the IgM antibody was heterospecific and cross reacted with one or more of the other dengue serotypes of JEV. There is no explanation for the predominantly monospecific IgM antibody in JEV infections, and the heterospecific IgM found with more regularity in dengue. The appearance of IgM antibody in these patients confirms the serological diagnosis of a primary dengue infection and suggests further, that patients under the age of one year can develop DHF or DSS after a primary dengue infection. One of these patients (CH-404k = #26, Fig 3) is presented in greater detail in the dengue section of this Annual Report.

#### D. Tropical fevers caused by Group B arbovirus.

Serial serum samples were obtained from a Peace Corps volunteer in Thailand before and after he developed a dengue-like febrile illness. He had been immunized against yellow fever before coming to Thailand and had received a primary and booster course of Biken killed JEV vaccine after his arrival. As shown in Table 7, results of HI tests were consistent with a recent 2° group B infection, virus type unspecified. The cross reactive IgM antibody in the convalescent serum suggests, more specifically, that he suffered a recent primary dengue virus infection.

MAJ Robert Howarth, 9th Med Lab, Vietnam, supplied 6 paired sera from U.S. troops in Vietnam hospitalized with fevers of unknown origin. Five of the 6 had serological evidence of a recent group B infection, with rising titers to JEV and to 3 or 4 dengue serotypes. One had high fixed titers to dengue 1-4 and JEV. These serological results together with IgM fractionation of the convalescent sera are shown in Table 8. JEV specific IgM antibody was found in 4 patients, and D1 specific antibody was detected in 1 patient. One patient was IgM negative, but his convalescent serum was drawn only 2 days after the acute phase serum. Thus IgM fractionation of convalescent serum in these FUO patients with 2° group B infections provided improved serological specificity, provided the convalescent serum was drawn at the proper time. No virus isolations are available to confirm these serological results, and the interpretation rests on serological similarity to previous virus confirmed patients together with the clinical and epidemiological data.

**SUMMARY AND CONCLUSIONS:** The fractionation and titration of serum IgM HAI antibody appears to be a new and useful serodiagnostic method in certain group B arbovirus infections. It is more virus specific than the standard serological tests, particularly in secondary infections, and often allows the diagnosis of a specific recent JEV or dengue infection at a time when the paired sera show only fixed elevated titers. The JEV IgM data for gibbons (this annual report) and previous human studies confirms that IgM antibody is produced after first JEV infection, apparent or inapparent, and after dengue, but not after a repeat infection with the same virus, a finding which has permitted certain seroepidemiological conclusions about group B arbovirus infections. For example, the presence of JEV-specific IgM antibody in serum of encephalitis patients showing nonspecific secondary group B antibody patterns (indicative of prior group B infection) suggests that their previous group B infection was not JEV and that there was no cross protection against the development of Japanese encephalitis in these patients.

The persistence of circulating IgM antibody for as long as 14 months after Japanese encephalitis suggests that the virus or noninfectious viral antigens may persist in some convalescing patients beyond the acute illness. This finding is of considerable theoretical interest in view of recent work elsewhere on slow virus infections. Another example of insight gained through IgM analysis concerns the 4 infant children studied with dengue hemorrhagic fever and shock syndrome. The detection of IgM antibody in convalescent sera of these children confirms the serological diagnosis of a primary dengue infection and thus suggests that children under 1 year can develop dengue complications following a primary infection rather than after the customary 2nd dengue infections as seen in older children.

The IgM technique has not proven useful in remote infections or when the convalescent serum cannot be dated with respect to the acute illness, owing to the transitory appearance of circulating IgM in most patients. Moreover the failure to detect IgM antibody by S-DGC, particularly in secondary infections, may result from technical insensitivity of the procedure (as described above) thereby giving a false negative result in the presence of low titered IgM antibody. Consequently the successful detection of IgM antibody has considerably more meaning than the failure to detect such antibody.

Table 1.  
JEV Serological Diagnosis Criteria\*

| <u>Response</u> | <u>Serum HI Antibody Titer</u>                 |  |
|-----------------|--|--|
|                 | <u>Acute</u>                                   | <u>Convalescent</u>  |
| Primary         | 1. $\leq 1:20$ to all dengue antigens.         | 1. $\geq 4$ fold rise to JE antigen<br>2. Titer to JEV $\geq 4$ fold titer to dengue 4<br>3. $\leq 1:80$ to dengue 4 antigen             |
| Secondary       | 1. $\geq 1:40$ to at least one dengue antigen. | 1. $\geq 4$ -fold rise to JEV and at least one dengue antigen.<br>2. Titer to JEV less than 4-fold greater than titer to dengue antigens |

\* Serum titered against Dengue 1-4 and JEV

Table 2.  
JEV-specific IgM antibody in JE patients listed by serological group, Chiangmai 1970.

| Whole serum<br>titer <sup>x</sup> | Total<br>pts. | Number of patients in whom: |                          |                  |           |
|-----------------------------------|---------------|-----------------------------|--------------------------|------------------|-----------|
|                                   |               | IgM antibody detected       |                          | IgM not detected |           |
|                                   |               | Primary <sup>xx</sup>       | Secondary <sup>xxx</sup> | Primary          | Secondary |
| Rising                            | 45            | 18                          | 15                       | 1                | 11        |
| Fixed                             | 6             | 1                           | 1                        | 0                | 4         |
| Falling                           | 3             | 0                           | 2                        | 0                | 1         |
| Totals                            | 54            | 19                          | 18                       | 1                | 16        |

x HI and/or CF

xx No serological evidence of previous group B arbovirus infection.

xxx Serological evidence of previous group B arbovirus infection.

Table 3.  
Cross Reactive IgM Antibody Patterns in Two Patients with Encephalitis

| Patient      | S-DGC*<br>Fraction | HI Titer in S-DGC Fractions |     |       |     |       |     |      |     |       |     |
|--------------|--------------------|-----------------------------|-----|-------|-----|-------|-----|------|-----|-------|-----|
|              |                    | JEV                         |     | D4    |     | D3    |     | D2   |     | D1    |     |
|              |                    | C                           | 2ME | C     | 2ME | C     | 2ME | C    | 2ME | C     | 2ME |
| JE-M-37      |                    |                             |     |       |     |       |     |      |     |       |     |
| 47 yr. woman | 3                  | 32                          | 8   | 16    | 8   | 16    | 8   | 16   | 4   | 32    | 4   |
| 6 Aug. 1970  | 4                  | 64                          | 8   | 16    | 8   | >128  | 8   | 64   | 4   | 64    | 4   |
|              | 5                  | 32                          | 8   | 16    | 8   | 16    | 8   | 16   | 4   | 16    | 4   |
| Whole        | 20 July 70         | 160                         |     | 320   |     | 320   |     | 160  |     | 160   |     |
| serum        | 6 Aug. 70          | 5120                        |     | 10240 |     | 20480 |     | 2560 |     | 10240 |     |
| JE-C-69      | 3                  | 16                          | 4   | 4     | 0   | 4     | 2   | 2    | 2   | 2     | 2   |
| 17 Aug. 70   | 4                  | 32                          | 4   | 8     | 0   | 4     | 2   | 2    | 2   | 2     | 2   |
|              | 5                  | 16                          | 4   | 4     | 0   | 4     | 2   | 2    | 2   | 2     | 2   |
| Whole        | 31 July 70         | 5120                        |     | 1280  |     | 1280  |     | 1280 |     | 640   |     |
| serum        | 17 Aug 70          | 2560                        |     | 640   |     | 1280  |     | 1280 |     | 640   |     |

\* sucrose density gradient centrifugation

Table 4.  
Longevity of IgM antibody in vivo after Japanese encephalitis

| Whole serum<br>antibody pattern <sup>x</sup> | Number of patients with IgM last detectable <sup>xx</sup><br>during month <sup>xxx</sup> |            |            |            |            |            |            |
|--|--|------------|------------|------------|------------|------------|------------|
|  | <u>0-1</u>   | <u>1-2</u> | <u>2-3</u> | <u>3-4</u> | <u>4-5</u> | <u>5-8</u> | <u>≥ 8</u> |
| Primary                                      | 11   |            | 4          | 3          |            | 1          |            |
| Secondary                                    | 10   | 4          | 2          |            |            | 1          | 1          |

x HI and/or CF

xx Values represent the minimum interval after illness

xxx After onset of illness

Table 5.  
Inapparent Infections (Confirmed & Questionable). Chiangmai Family Case Members — 1970.  
Sera fractionated by S-DGC for IgM Ab vs. D 1-4, JE, Tembusu, Wesselsbron.

| Pt. No./<br>HI pattern <sup>xx</sup> | Serum<br>No. | Date    | S-DGC<br>Results | Antigens*      |
|--------------------------------------|--------------|---------|------------------|----------------|
| JE-C-6c<br>1°-R                      | 44585        | 25 May  | N.T. #           |                |
|                                      | 44586        | 9 June  | N.T.             |                |
|                                      | 46037        | 14 June | IgM + JEV        | JE, D4, T, W   |
| JE-L-121<br>1°-R                     | 44709        | 11 June | N.T.             |                |
|                                      | 44710        | 23 June | IgM + JEV        | JE, D4, T, W   |
| JE-L-11a<br>1°-R                     | 44762        | 15 June | N.T.             |                |
|                                      | 44763        | 29 June | IgM + JEV        | JE, D4, T, W   |
| JE-C-26D<br>1°-R                     | 44966        | 29 June | N.T.             |                |
|                                      | 44967        | 13 July | IgM + JEV        | JE, T, D4      |
|                                      | 46049        | 19 Aug  | IgM ± JEV        | JE, T, D4, W   |
| JE-C-32c<br>2°-R                     | 45177        | 2 July  | N.T.             |                |
|                                      | 45178        | 17 July | IgM Neg.         | JE, D1-4, T, W |
|                                      | 46206        | 21 Aug  | IgM Neg.         | JE, D4, T      |
| JE-M-53d<br>1°-R                     | 46635        | 30 Sept | IgM Neg.         | D4, JE, T, W   |
|                                      | 46636        | 12 Oct  | N.T.             |                |
|                                      | 46869        | 12 Nov  | IgM Neg.         |                |

# N.T. = not tested

\* D4 = dengue 4, D1-4 = Dengue 1-4, T = Tembusu, W = Wesselsbron

xx 1°-R = monospecific rising titer to JEV (see Table 1)

2°-R = heterospecific rising titers to group B antigens (see Table 1)

Table 6.  
IgM antibody pattern in infants with dengue hemorrhagic fever or shock syndrome -- 1971

| Patient                | S-DGC fraction | HI titer in S-DGC fractions |            |          |            |          |            |          |            |          |            |
|------------------------|----------------|-----------------------------|------------|----------|------------|----------|------------|----------|------------|----------|------------|
|                        |                | JEV                         |            | D4       |            | D3       |            | D2       |            | D1       |            |
|                        |                | <u>C</u>                    | <u>2ME</u> | <u>C</u> | <u>2ME</u> | <u>C</u> | <u>2ME</u> | <u>C</u> | <u>2ME</u> | <u>C</u> | <u>2ME</u> |
| CH-404K                | 2              | 4                           | 0          | 4        | 0          | 2        | 0          | 8        | 0          | 2        | 0          |
| 49133                  | 3              | 8                           | 0          | 16       | 0          | 8        | 0          | 16       | 0          | 4        | 0          |
| D2 isolated            | 4              | 2                           | 0          | 4        | 0          | 4        | 0          | 4        | 0          | 0        | 0          |
| CH-408K                | 2              | 2                           | 0          | 4        | 0          | 2        | 0          | 16       | 0          | 0        | 0          |
| 49100                  | 3              | 4                           | 0          | 16       | 0          | 8        | 0          | 32       | 2          | 2        | 0          |
| virus neg.             | 4              | 2                           | 0          | 4        | 0          | 4        | 0          | 16       | 2          | 2        | 0          |
| CH-454M                | 2              | 2                           | 0          | 4        | 0          | 2        | 0          | 16       | 0          | 2        | 0          |
| 49664                  | 3              | 8                           | 0          | 16       | 2          | 8        | 0          | 32       | 2          | 4        | 0          |
| virus neg.             | 4              | 2                           | 0          | 4        | 2          | 4        | 0          | 16       | 2          | 2        | 0          |
| RA-005K                | 2              | 2                           | 4          | 4        | 2          | 2        | 0          | 8        | 0          | 2        | 0          |
| 48709                  | 3              | 4                           | 0          | 8        | 2          | 4        | 0          | 32       | 0          | 4        | 0          |
| No isolation attempted | 4              | 2                           | 0          | 4        | 2          | 2        | 0          | 8        | 0          | 2        | 0          |

Table 7.  
Whole serum and IgM HI Antibody in a Peace Corps Volunteer with a Febrile Dengue-Like Illness

| S-DGC fraction          | HI titer in S-DGC fractions of convalescent serum |            |          |            |          |            |          |            |          |            |
|-------------------------|---|------------|----------|------------|----------|------------|----------|------------|----------|------------|
|                         | JEV   |            | D4       |            | D3       |            | D2       |            | D1       |            |
|                         | <u>C</u>  | <u>2ME</u> | <u>C</u> | <u>2ME</u> | <u>C</u> | <u>2ME</u> | <u>C</u> | <u>2ME</u> | <u>C</u> | <u>2ME</u> |
| 2                       | 8   | 2          | 4        | 4          | 4        | 2          | 8        | 0          | 2        | 0          |
| 3                       | 16  | 4          | 16       | 8          | 8        | 2          | 32       | 2          | 4        | 0          |
| 4                       | 8   | 4          | 8        | 8          | 8        | 2          | 16       | 2          | 4        | 0          |
| whole acute serum conv. | <10   |            | <10      |            | <10      |            | <10      |            | <10      |            |
|                         | 640   |            | 2560     |            | 320      |            | 640      |            | 320      |            |

Table 8.  
Whole Serum and IgM HI Antibody in American Troops Hospitalized  
with Tropical Fevers, Vietnam 1970

| Patient        | Date serum | HI titers in whole serum vs |      |      |      |      |      | IgM Antibody detected <sup>x</sup> vs |
|----------------|------------|-----------------------------|------|------|------|------|------|---------------------------------------|
|                |            | JEV                         | D4   | D3   | D2   | D1   | Chik |                                       |
| Jackson        | 4/10/70    | 0                           | 0    | 0    | 0    | 0    | 0    | JEV                                   |
|                | 18/10/70   | 320                         | 80   | 80   | 40   | 40   | 0    |                                       |
| Christopherson | 2/11/70    | 0                           | 0    | 0    | 0    | 0    | 0    | not detected                          |
|                | 4/11/70    | 160                         | 20   | 40   | 20   | 0    | 0    |                                       |
| Bartsch        | 22/11/70   | 160                         | 40   | 40   | 20   | 20   | 0    | JEV                                   |
|                | 29/11/70   | 10240                       | 1280 | 2560 | 640  | 320  | 0    |                                       |
| Hanson         | 17/9/70    | 80                          | 0    | 20   | 0    | 0    | 0    | JEV                                   |
|                | 29/9/70    | 640                         | 80   | 160  | 40   | 40   | 0    |                                       |
| Shire          | 19/11/70   | 5120                        | 5120 | 2560 | 640  | 1280 | 0    | Dengue—1                              |
|                | 22/11/70   | 10240                       | 1280 | 5120 | 1280 | 1280 | 0    |                                       |
| Kokanich       | 20/11/70   | 2560                        | 320  | 640  | 160  | 80   | 0    | JEV                                   |
|                | 2/12/70    | > 20480                     | 640  | 1280 | 640  | 320  | 0    |                                       |

x IgM titered against D1-4 & JEV.