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**Short Communication**

# A Case of Chikungunya Fever Imported from India to Japan, Follow-Up of Specific IgM and IgG Antibodies over a 6-Month Period

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**SUMMARY**: Chikungunya fever is an arboviral disease caused by chikungunya virus. A 37-year-old Japanese male visited India and developed fever, myalgia, rash, and persisting systemic arthralgia, the latter of which persisted for more than 2 months. The patient was diagnosed with chikungunya fever by virological and serological examinations. In the present study, we followed specific antibody responses over a 6-month period after the onset of the disease. IgM antibody was detected on days 58 and 108, but not on day 137, by enzyme-linked immunosorbent assay. Specific IgG and neutralizing antibodies were detected as late as day 192. The results indicate that specific IgM lasts for 3 to 4 months from the onset of the disease, and that IgG lasts more than 6 months.

Chikungunya (CHIK) fever is an acute febrile illness caused by mosquito-borne chikungunya virus (CHIKV) that belongs to the genus alphavirus of the family *Togaviridae*. The main symptoms of CHIK fever are high temperature, arthralgia, headache and myalgia, and occasional rash. Polyarthralgias or arthritis may last for months to years (1). CHIKV was first isolated in Tanzania in 1952 (2). CHIKV is currently distributed in Africa and in South and Southeast Asia (3). Outbreaks have occurred periodically in these regions for the past 50 years at 7- to 20-year intervals (4). In 2004–2006, a large CHIKV outbreak occurred in islands in the western Indian Ocean: Comoros, Mayotte, Mauritius, the Seychelles, and Réunion Island (5). Outbreaks of CHIK fever have recently occurred in India (3), Sri Lanka, and then in the Southeast Asian countries of Singapore, Malaysia (6), Indonesia, and Thailand. Another important issue has been in the spread of CHIK fever cases from epidemic areas to Europe (7), Canada, the Caribbean, South America, and the United States (8). Further, an outbreak occurred in Italy, originating from a patient returning from India. Approximately 300 patients with one fatality were reported in this outbreak (9). There have been imported CHIK fever cases in Japan. In the present study, we followed antibody responses in a CHIK fever patient who had just returned from India.

A 37-year-old male visited India on business from July 16 to August 8, 2008. On July 26, he developed a high fever (39.0°C), headache, and generalized arthralgia, and was admitted to a hospital. He was treated with antibiotics for 4 days. The fever had subsided by July 29. The next day, a generalized spotted rash developed on his entire body except for his face, and lasted for several days. He was diagnosed with CHIK fever based on the clinical symptoms. He tested negative for CHIKV-specific antibodies. He was discharged from the hospital on August 1 and returned to Japan on August 8. His arthralgia became exacerbated in the middle of August. The patient visited the Osaka City General Hospital on September 22.

The chief complaint at the consultation was a sense of incongruity at the right wrist, proximal interphalangeal joints, the right knee, and the joints in his left foot. On physical examination, swelling and deformity were not present in the systemic joint. The results of the blood test on September 22 were hemoglobin 17.8 g/dl, total leukocyte count 11,200/$\mu$l, platelet count 250,000/$\mu$l, aspartate aminotransferase (AST)/alanine aminotransferase (ALT) 52/89.

Virological examinations were performed using the serum specimen collected on September 22 (day 58 after onset of the disease) (Table 1). CHIKV-specific IgM and IgG antibodies were positive by indirect immunofluorescence assay (IFA). CHIKV-specific IgM was also positive by enzyme-linked immunosorbent assay (ELISA) (P/N ratio was 3.88 [>2.0 to be positive]). Neutralizing antibody titers to CHIKV were 1:160 by the plaque-reduction neutralization test (50% PRNT). IgM and IgG antibodies to dengue and Japanese encephalitis viruses were negative, determined by IFA and ELISA. Reverse transcription-polymerase chain reaction (RT-PCR) was performed with CHIKV-specific primer (CHIKnsp1-S/nsp2-C, CHIK/E1-S/E1-C [10], and Chik10294s/10573c), and flavivirus-specific primer (YF1/YF3) (11). The viral RNAs were negative by RT-PCR. The diagnosis of CHIK fever was confirmed on the basis of these laboratory data, the presence of CHIKV-specific IgM and IgG antibodies in the convalescent phase of the disease, and the absence of CHIKV-specific antibody in the acute phase of the disease in India.

To follow the duration of IgM and IgG antibodies to CHIKV, blood specimens were collected every other month, on days 108, 137, 164, and 192, and examined for the presence of IgM and IgG antibodies by ELISA, IFA, and 50% PRNT (Table 2). CHIKV-specific IgM antibody was positive on day 108 but negative on day 137. Neutralizing and IFA IgG antibodies were positive at the same titers throughout the examined period.

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Table 1. Results of the virological examinations of the patient serum collected on September 22 (58 days after onset of the disease)

| Virus | Test item | Method | Result |
|---|---|---|---|
| Chikungunya virus | IgM | IFA | 20 |
| | IgM | ELISA | positive |
| | IgG | IFA | 160 |
| | Neutralizing antibody | NT | 160 |
| | nsP1–nsP2, E1 gene | RT-PCR | negative |
| Dengue virus | IgM, IgG | IFA | negative |
| | IgM, IgG | ELISA | negative |
| Japanese encephalitis virus | IgM, IgG | IFA | negative |
| Flavivirus | NS5-3′UTR gene | RT-PCR | negative |

RT-PCR, reverse transcription-polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; IFA, immunofluorescent assay; NT, 50% plaque-reduction neutralization test.

Table 2. Change of antibody titer to CHIKV of the patient

| Days after onset of the disease | ELISA | IFA | | NT |
|---|---|---|---|---|
| | IgM | IgM | IgG | |
| 58 | positive | 20 | 160 | 160 |
| 108 | positive | <10 | 160 | 320 |
| 137 | negative | <10 | 160 | 320 |
| 164 | nt | nt | 160 | 320 |
| 192 | nt | nt | 160 | 320 |

Abbreviations are in Table 1.

It should be noted that the CHIKV-specific IgM was positive by ELISA for 108 days. This suggests that CHIKV-specific IgM antibody can be used to diagnose CHIKV infection even 3 to 4 months after the onset of the disease, especially for those who do not receive confirmatory diagnoses in the endemic countries. A recent report demonstrated that IgM antibody lasted for 1 year in the CHIK fever patients with cryoglobulinemia (12). On the other hand, these results suggest that the presence of IgM to both dengue virus and CHIKV does not directly support dual infection.

The first and second imported CHIK fever cases were confirmed in November 2006 among returnees from Sri Lanka (13,14). The patient in the present report is the third imported CHIK fever case in Japan. Another 11 imported cases have been subsequently confirmed: 2 from India, 6 from Indonesia, 2 from Malaysia, and 1 from Thailand (Takasaki et al., personal communication. Online at <http://www.nih.go.jp/vir1/NVL/Aiphavirus/Chikungunyahtml.htm> [in Japanese]). The main vector mosquito of CHIKV is *Aedes albopictus*, which inhabits most regions of Japan (15–17). Thus, there is a possibility that an outbreak of CHIK fever originated from these imported cases in Japan, which was the same situation in Italy.

CHIK fever is not designated as a reportable disease in the Infectious Disease Control Law or in the Quarantine Act at present in Japan. CHIK fever has symptoms similar to those of other mosquito-borne diseases such as dengue fever and West Nile fever. CHIK fever should be included as a differential diagnosis for patients returning from South and Southeast Asian countries, and from islands and countries located on the Indian Ocean. Detection of IgM is a key laboratory diagnostic technique for CHIK fever.

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