NOTES

Newly Isolated Strains of Rickettsia tsutsugamushi in Japan
Identified by Using Monoclonal Antibodies to Karp, Gilliam, and
Kato Strains

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Four isolated strains of Rickettsia tsutsugamushi from patients in a new endemic area of Japan were tested
for antigenicities by using 12 monoclonal antibodies to Karp, Gilliam, and Kato strains. It was suggested that
one isolate was Karp related and that the others were two independent strains.

Since 1976, the incidence of tsutsugamushi disease has increased in Japan (9). It had never been reported in Gifu
Prefecture, located near the center of Japan’s main island of Honshu, until 1981. After 1982, the recorded incidence of
the disease in this prefecture was 5th to 7th highest (26 to 52
patients per year) in Japan. We isolated four strains of
Rickettsia tsutsugamushi from patients in this area to investi-
gate the nature of these strains, not only to detect the
prevalent strains in this area but also to speculate on the real
cause of the sudden emergence of the disease in this prefecture. Recent studies suggested the usefulness of murine
monoclonal antibodies (MAbs) to standard strains (Karp,
Gilliam, and Kato) of R. tsutsugamushi for classifying the
isolates (1, 2, 7). In this study, the antigenicities of the new
isolates were analyzed by using either strain-specific or
cross-reactive MAbs to standard strains.

The Karp, Gilliam, and Kato strains of R. tsutsugamushi
were kindly supplied from the Toyama Prefectural Institute
of Public Health, Toyama, Japan. Four newly isolated strains
from tsutsugamushi disease patients in Gifu Prefecture
(Japan) (5) were propagated in BS-C-1 cells. Maintenance
of these strains was performed as described by Tamura et al. (10).

Five- to six-week-old male BALB/c mice were infected
either subcutaneously (for Karp and Kato strains) or intra-
peritoneally (for Gillian strain) with 100 times the mouse
50% lethal dose of each strain. After 5 to 6 weeks, the mice
were inoculated intravenously with 1,000 times the mouse
50% lethal dose of the same rickettsial strain. Lymphocyte
hybridomas were prepared 7 days later by fusing the spleen
and NS-1 myeloma cells by using polyethylene glycol (Koch-
Light Laboratories Ltd., Colnbrook, England), as described
by others (6, 8). Hybridomas secreting rickettsial antibodies
were identified by using the indirect fluorescent-antibody
test (IFA) in which culture fluids were reacted with spotted
infected cells and fluorescein isothiocyanate-labeled anti-
mouse immunoglobulin G (heavy and light chain) goat serum
(Cooper Biomedical, Inc., West Chester, Pa.). These hybrid-
omas were cloned two to three times by limiting dilutions.
Rickettsial antibody-secreting hybridomas (107 cells) were
injected into pristane (Aldrich Chemical Co., Inc., Madison,
Wis.)-primed BALB/c nude mice. The immunoglobulin class
of the MAb was determined by the Ouchterlony method
using anti-μ, anti-γ1, anti-γ2a, anti-γ2b, and anti-γ3 serum
(Cooper Biomedical). The immune ascites were titrated to
standard and isolated strains of R. tsutsugamushi by IFA.

Twelve clones of hybridomas that secreted MAbs to standard strains of R. tsutsugamushi were established. The
reactivity of each MAb is shown in Table 1. Strain-specific
MAbs (Kp/D11, Gi/E4, Kt/1D2, and Kt/2D9) were identified
by IFA titers against homologous antigens ranging from
1/320 to 1/20,480 and titers of less than 1/10 against heterologous antigens. Two MAbs (Kp/C6 and Kt/3B2) exhibited
similar IFA titers with all three standard strains. Kp/1C10
exhibited IFA titers of 1/1,280 against a homologous strain
and 1/80 against heterologous strains. Five MAbs reacted
with a homologous strain plus one of the others, i.e., three
MAbs (Kp/1F11, Kt/4D9, and Kt/3C2) reacted with both
Karp and Kato strains. One MAb (Kp/2B7) reacted with
both Karp and Gilliam strains, and another (Gi/E2) reacted with both Gilliam and Kato strains.

Four isolated strains (KN-1, KN-2, KN-3, and KN-4) were examined for reactivities to 12 MAbs by IFA to classify
their antigenicities (Table 1). Strain KN-3 reacted with
Karp-specific Kp/D11 and Karp-Kato-reactive Kp/1F11 and
Kt/3C2. However, strain KN-3 reacted with neither Karp-
KN-1 was the only strain which did not react with cross-
reactive Kp/1C10. Furthermore, strain KN-1 reacted slightly
with Karp-specific Kp/D11 and Gilliam-specific Gi/E4 but
not with Kato-specific MAb. Strains KN-2 and KN-4 were
suspected of being identical because they exhibited almost
the same titers against all MAbs tested. These strains had
reactivities to cross-reactive MAbs (Kp/1F11, Kp/1C10, Kp/
C6, and Kt/3B2) with high titers and Karp-specific and
Kato-specific MAbs (Kp/D11 and Kt/1D2) with low titers.

The usefulness of MAbs to R. tsutsugamushi strains for
classifying their antigenicities was suggested by Eisemann
and Osterman (1), Murata et al. (7), and Kanemitsu (2). In
the present study, we established 12 strain-specific and

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cross-reactive MAb s to standard strains of *R. tsutsugamushi*. We were able to identify four newly isolated strains (KN-1, KN-2, KN-3, and KN-4). These isolates fell into three groups by using the IFA with MAb s, and only one (KN-3) was related to the Karp strain. Strain KN-3 differed from the Karp strain, however, by having low reactivity (Kp/C6) and nonreactivity (Kp/2B7 and Kt/4D9) with MAb s to the Karp strain reacted with high titers. These results suggested that KN-3 was an independent strain, although it is possible that the isolated strain or KN-3 differed from the laboratory-maintained strain only by epitope density. Strains KN-1 and KN-2 (=KN-4) were suggested to be independent strains because of low or no reactivities against strain-specific MAb s. Moreover, reactivity against cross-reactive Kp/1C10 was critical between the two strains.

In recent years, several investigators reported new isolates which were antigenically distinguished from standard strains (2, 5, 11). Kobayashi et al. (4) reported newly isolated strains which were identified as the Gilliam-Kato type by using the IFA technique with MAb s to Karp, Gilliam, and Kato strains. It is surprising to see that three independent strains were isolated from four patients in such a small geographical area and that all isolates revealed antigenic differences from standard strains.

Further investigation should focus on an analysis of the antigenicities of these isolated strains, along with their pathogenicities.

**LITERATURE CITED**


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**TABLE 1. Reactivities of MAb s to standard and isolated *R. tsutsugamushi* strains**

<table>
<thead>
<tr>
<th>Clone code</th>
<th>Immunoglobulin</th>
<th>Standard strains</th>
<th>IFA titer&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Isolated strains</th>
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<tr>
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<td>M</td>
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<td>&lt;&lt;</td>
<td>20</td>
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<tr>
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</tbody>
</table>

<sup>a</sup> Reciprocal of the highest dilution of immune ascitic fluid causing rickettsial fluorescence.

<sup>b</sup> <, Less than 1:10.