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Characterization of a New Antigenic Type, Kuroki, of *Rickettsia tsutsugamushi* Isolated from a Patient in Japan

NORIO OHASHI,[1] AKIRA TAMURA,[1]* HIDEYUKI SAKURAI,[1] AND SEIGO YAMAMOTO[2]

Department of Microbiology, Niigata College of Pharmacy, 5-13-2 Kamishin'ei-cho, Niigata 950-21,[1] and Miyazaki Prefectural Public Health Laboratory, Miyazaki 889-21,[2] Japan

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The Kuroki strain of *Rickettsia tsutsugamushi*, isolated from a patient in Kyushu, Japan, has a major, type-specific antigenic polypeptide which is distinct from the prototype strains in size (58 kilodaltons), in antigenicity, and in its cleavage pattern with *N*-chlorosuccinimide. These results indicate that Kuroki is another antigenic type of *R. tsutsugamushi*.

Rickettsia tsutsugamushi is unique among *Rickettsia* spp. because of the presence of several antigenic variants (2, 9, 11, 13) and the lack of peptidoglycan and lipopolysaccharide (1). Of the antigenic variants, rickettsiae of Gilliam, Karp, and Kato types (prototype strains) are commonly isolated from patients in Japan and the other endemic areas. The other antigenic types were first recognized in Thailand by Elisberg et al. (2), and we also found in Japan that Shimokoshi (9) and Kawasaki (13) strains, which were isolated from patients in Niigata and Miyazaki prefectures, respectively, showed antigenicities distinct from those of the prototype strains. Our previous studies demonstrated that this antigenic diversity depends on a type-specific antigen of a 54- to 56-kilodalton (kDa) polypeptide located on the rickettsial surface (5–8, 13). Recently, Yamamoto and coworkers (12) suggested the prevalence of another new antigenic type, Kuroki, in Kyushu, from the cross-reactivities with patient sera in immunofluorescence tests, and they showed that almost all isolates from patients in the endemic area are classified into two antigenic types—Kawasaki and Kuroki. In this report, Kuroki is confirmed as a new antigenic type of *R. tsutsugamushi* on the basis of comparative studies of protein composition and antigenic components among antigenically distinct strains.

R. tsutsugamushi was propagated in a suspension culture of L929 cells and purified by Percoll density gradient centrifugation (10). Guinea pig hyperimmune sera were prepared by intracerebral inoculation of the rickettsiae (8). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were performed as described previously (8). Cleavage of the type-specific antigen with *N*-chlorosuccinimide was performed by the techniques of Lischwe et al. (3). Briefly, purified rickettsiae were solubilized and electrophoresed by SDS-PAGE (10% polyacrylamide gel). Then, the banding areas of type-specific antigens on the gel were cut out of the gel, washed twice with water and twice with 1 g of urea–1 ml of H_2O–1 ml of CH_3COOH, incubated in 1 mg of *N*-chlorosuccinimide per ml in urea-H_2O-CH_3COOH for 30 min, and washed twice with water, followed by equilibration in sample SDS-PAGE buffer for 1.5 h and loading on a resolving gel for SDS-PAGE (12.5% gel). After electrophoresis, the cleaved polypeptides were observed by double staining with Coomassie brilliant blue and silver (4). The molecular weight of each polypeptide was estimated by comparison with the migration distances of standard proteins: phosphorylase *b* (97,400), glutamate dehydrogenase (55,400), lactate dehydrogenase (36,500), trypsin inhibitor (21,500), and cytochrome *c* (12,500) (Boehringer Mannheim Biochemicals, Mannheim, Federal Republic of Germany).

Comparison of the polypeptide compositions of Kuroki, Gilliam, Karp, Kato, Shimokoshi, and Kawasaki strains by SDS-PAGE revealed the similarity of overall patterns among the strains (Fig. 1). However, the most major polypeptide in the Kuroki strain was located at the 58-kDa position, which is slightly higher than those of the corresponding polypeptides of the other strains (54 to 56 kDa). The bands of 46, 60, and 70 kDa in the Kuroki strain were observed at positions similar to those of the corresponding bands of the other strains.

In immunoblotting analyses (Fig. 1), guinea pig hyperimmune sera against Gilliam, Karp, Kato, Shimokoshi, and Kawasaki strains (Fig. 1) reacted clearly with the 54- to 56-kDa polypeptides of the homologous strains but weakly with the 58-kDa polypeptide of the Kuroki strain. On the other hand, anti-Kuroki serum reacted strongly with the 58-kDa polypeptide of the Kuroki strain but weakly or not at all with the 54- to 56-kDa polypeptides of the other strains. These results indicate the strain-specific antigenicity of these major polypeptides as mentioned previously (8) and suggest that Kuroki strain is antigenically distinct from the other five strains.

The cleavage patterns of the type-specific antigens (54- to 58-kDa polypeptides) of the six strains with *N*-chlorosuccinimide were different from each other in SDS-PAGE (Fig. 2). The Kuroki strain was especially distinguishable from the others in its overall patterns and in the mobility of its individual fragments; i.e., the Kuroki strain revealed eight bands between 19 and 43 kDa, while the other strains showed five to six bands in this area. These results indicate the differences of the molecular structures of the strain-specific antigens among the antigenic variants, especially in the Kuroki strain. A small amount of the 60-kDa polypeptide may have contaminated the 54- to 58-kDa polypeptide fraction, but it would not have affected the patterns, because we

* Corresponding author.

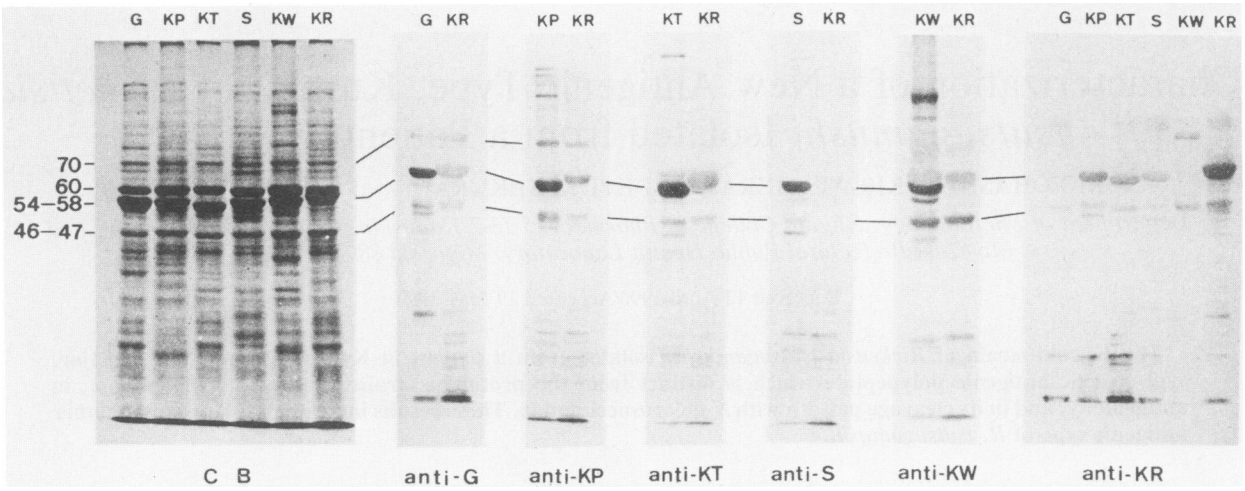

FIG. 1. SDS-PAGE patterns and immunoblotting profiles of antigenically distinct strains of *Rickettsia tsutsugamushi*; G, Gilliam; KP, Karp; KT, Kato; S, Shimokoshi; KW, Kawasaki; KR, Kuroki. The six columns at the left were stained with Coomassie blue (CB). The others were blotted with guinea pig hyperimmune sera against each strain indicated at the bottom. Numbers indicate the molecular sizes, in kilodaltons, of the bands.

confirmed in a preliminary test that the 60-kDa polypeptides are not cleaved under the conditions we used.

Our previous studies (9, 13) demonstrated that the Shimokoshi and Kawasaki strains are antigenically distinct from the prototype strains of Gilliam, Karp, and Kato. The present study shows that the Kuroki strain represents another antigenic type in Japan. Our recent observations (12) indicate that the rickettsiae isolated from patients with scrub typhus in Miyazaki prefecture are all classified as either Kawasaki or Kuroki type but that almost all rickettsiae isolated in Niigata prefecture are classified as either Gilliam or Karp type. The incidence of isolations of Shimokoshi and Kato types is very low. This suggests that there are regional distributions of the serotypes of *R. tsutsugamushi* in each endemic area and that the strains of Kuroki type may be distributed in southern Japan.

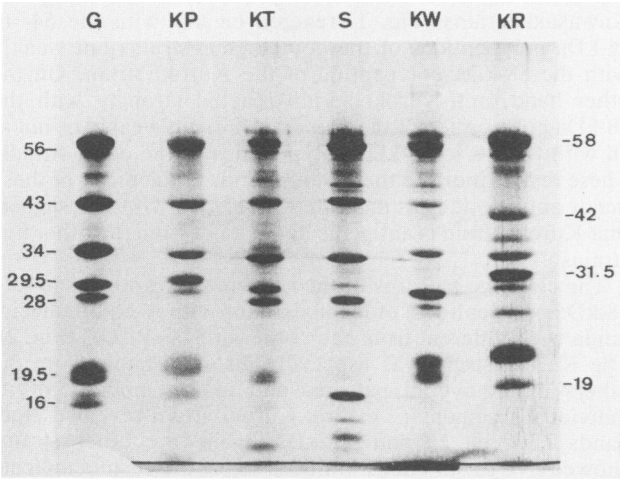

FIG. 2. Cleavage patterns of the major polypeptides (54 to 58 kDa) of each strain with *N*-chlorosuccinimide. Numbers indicate approximate molecular sizes (in kilodaltons) of the peptide fragments from Gilliam (left) and Kuroki (right) strains. Abbreviations at the top of each column are the same as for Fig. 1.

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