JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1997, p. 261–263 Vol. 35, No. 1
0095-1137/97/$04.00+0
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Protein Characterization of Australian Spotted Fever Group Rickettsiae and Monoclonal Antibody Typing of *Rickettsia honei*

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Received 8 August 1996/Returned for modification 28 August 1996/Accepted 1 October 1996

Rickettsial proteins rOmp A and rOmp B exist in both *Rickettsia australis* and *Rickettsia honei* but differ in molecular weight and antigenicity; in addition, they produce distinct immunogenic responses and appear to be to conformationally dependent antigens. Species-specific monoclonal antibodies for other spotted fever group rickettsial species did not react with *R. honei*. A PCR product of the repeat region of the rOmp A gene from *R. honei* was amplified and calculated to contain 11 repeat units.

There are two rickettsial spotted fever group (SFG) diseases that are currently recognized in Australia. The first, commonly known as Queensland tick typhus (QTT), is caused by *Rickettsia australis* and occurs along the east coast of Australia (3, 5, 12, 20). The second is found on Flinders Island, which is located in Bass Strait, neighboring Tasmania, and is recognized as Flinders Island spotted fever (FISF) (19). An SFG rickettsiosis of unknown etiology has also been detected in Tasmania (4, 10). The clinical symptoms of these diseases are similar, displaying the classical features of SFG rickettsial ailments. The majority of patients presented with malaise, myalgia, headache, fever, eschar, maculopapular rash, and lymphadenopathy (17). Primary isolations of rickettsial agents were made from buffy coat-enriched blood from patients presenting with the symptoms of FISF, and the two isolates were designated RB and RM (10).

A number of Australian SFG rickettsial strains were partially characterized by sequencing of the 17-kDa-antigen gene, and several differences between the QTT and FISF agents were noted (1). On the basis of these results, the name *Rickettsia honei* has been proposed for the FISF agent (1). The present study characterized the proteins of the two Australian rickettsial SFG species by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting.

Rickettsiae were cultivated in Vero cell cultures and purified as described previously (1, 11). The antigens were dissolved in sample buffer (13), either at room temperature or at 100°C for 5 min. Since *R. honei* strains displayed similar immunoblot results (data not shown), the RB strain was chosen as the reference strain for comparisons with the *R. australis* PHS strain.

Standard immunoblotting and Coomassie blue staining techniques were used following SDS-PAGE (2, 9, 16). Analysis of *R. australis* and *R. honei* lysates by SDS-PAGE revealed differences in the migration of the Coomassie blue-stained proteins (Fig. 1). Although some protein bands of *R. australis* and *R. honei* have similar electrophoretic mobilities, there are several which seem particular to each species. The heat-denatured proteins displayed migratory patterns differing from those of the proteins dissolved at room temperature, with protein bands becoming more clearly defined in the 30- to 50-kDa range. The two predominant higher-molecular-mass proteins are likely to be rOmp A and rOmp B, which are observed with other SFG species. For example, two well-characterized immunodominant high-molecular-mass proteins from *R. rickettsii* known as rOmp A and rOmp B migrate with molecular masses of 190 and 135 kDa, respectively, after heat denaturation (22). Comparisons of the *R. australis* and *R. honei* lysates showed an apparently greater molecular weight change between the electrophoretic mobilities of the rOmp A molecules upon heating than for rOmp B. The rOmp B gene has been shown to be conserved among the SFG and typhus group rickettsiae (8), whereas the rOmp A protein is absent from *Rickettsia prowazekii* and *Rickettsia typhi* and seems to be more variable, mostly owing to differences in the number, order, and possibly absence of the repeat units in different species and strains (7).

Most of the monoclonal antibodies (MAbs) used in this study have been described previously (14, 15, 21, 23) (Table 1) with the exception of MAbs 16E11, 2D2-D5, GE10-H8, and 1F3-G3, which were prepared as described previously (23). MAb GE10-H8 reacts with rOmp A, and MAb 1F3-G3 reacts with rOmp B of many SFG rickettsial species. Reactivity of these rickettsial species with rOmp A- and rOmp B-specific MAbs identified the rOmp A and rOmp B of *R. honei* (Fig. 2), but there was no cross-reactivity with the *R. australis* proteins. The apparent molecular masses appeared to be 130 kDa for rOmp A and 125 kDa for rOmp B from the nondenatured *R. honei* lysates; with heat denaturation, these proteins migrated at 134 and at 125 kDa, respectively. In the nondenatured state, the two high-molecular-mass immunoreactive proteins of *R. australis*, the putative rOmp A and rOmp B, have apparent molecular masses of 140 and 115 kDa, respectively (Fig. 1).

Species-specific MAb typing of *R. honei* revealed no reactivity with any of the species-specific antibodies tested. Although these results are not conclusive regarding the uniqueness of this organism, they illustrate further differences between *R. honei* and other pathogenic SFG rickettsial antigenic markers (Table 1).

Immunoblot analysis of *R. australis* and *R. honei* with sera from patients with FISF and QTT demonstrated species differences in reactivity (Fig. 3). Although there was some reactivity of the FISF serum with *R. australis* and of the QTT serum with *R. honei*, the predominant reactivity was with the homol-

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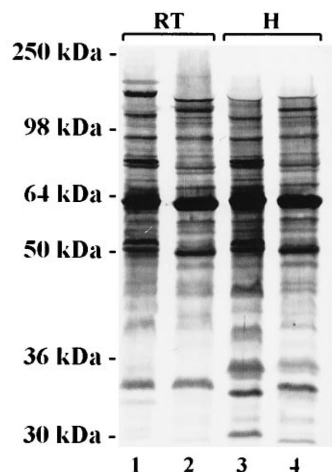

FIG. 1. Coomassie stained, SDS–10% PAGE gel of rickettsial lysates. *R. australis* (lanes 1 and 3) and *R. honei* (lanes 2 and 4) were dissolved at room temperature (RT) or by heating (H) for 5 min.

ogous species. This analysis demonstrated the conformational nature of the antigens of the two high-molecular-mass proteins as revealed by the absence of immunoreactivity after heating of the protein samples prior to electrophoresis.

The specific reactivity was also reflected in the results of testing acute-phase and convalescent-phase serum samples for the presence of antirickettsial immunoglobulin G (IgG) and IgM by immunoblotting (Fig. 4). QTT and FISF patient sera reacted with both agents to some degree. The IgM response of acute-phase sera was primarily directed against the two highest-molecular-weight proteins and the lipopolysaccharide component. The convalescent-phase sera showed an increase in the intensity of the IgM response, including increased reactivity with the midrange proteins (30 to 80 kDa). Although the IgG response was weak in both acute- and convalescent-phase sera, the reactivity was predominantly against the two higher-molecular-weight proteins, the LPS and a 64-kDa protein.

The PCR amplification of the repeat domain of the rOmp A gene (23) from *R. honei* yielded a 2.4-kb product which is estimated to encode 11 repeat units (data not shown). Attempts at PCR amplification of this region from *R. australis* yielded no DNA product; other reported attempts at amplifying segments of the rOmp A gene from *R. australis* have also failed (6, 8, 24). This result provides further evidence that *R. honei* is more closely related to the other SFG rickettsiae than to *R. australis*.

Traditionally, the SFG members were assigned species according to the geographic limits of a particular disease, such as

TABLE 1. Species-specific MAbs obtained from mice immunized with SFG rickettsiae and found to be nonreactive against *R. honei* antigens

MAb	Species specificity	Reference
F3-30	*R. rickettsii*	17
16E11	*R. rickettsii*	Unpublished data
77-224-20	*R. akari*	18
3Y6-C1	*R. japonica*	19
U 12	*R. conorii*	16
4B4	*R. conorii* (Israel 293)	16
F2-61	*R. conorii*	17
U 11	*R. conorii*	16
U 14	*R. conorii*	16
U 20	*R. conorii*	16
6 D11 H2	*R. sibirica*	16
2D2-D5	*R. sibirica*	Unpublished data

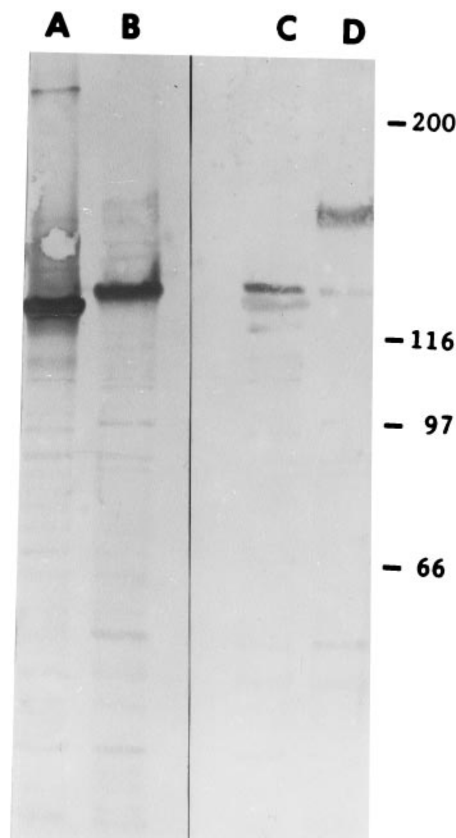

FIG. 2. Western immunoblot of nondenatured antigens of *R. honei* (lanes A and C) and *R. rickettsii* (lanes B and D) reacted with MAbs to rOmp B (lanes A and B) and rOmp A (lanes C and D). Numbers on the right are molecular masses, in kilodaltons.

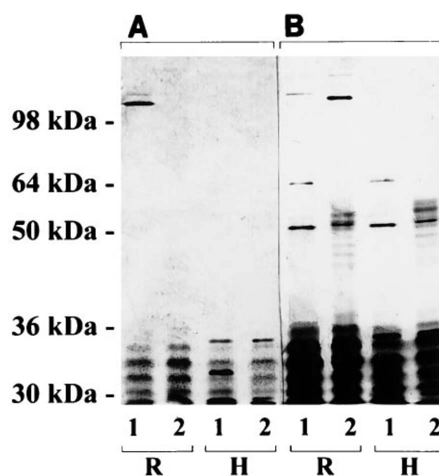

FIG. 3. Immunoblot of SFG patient sera. *R. honei* (lanes 1) and *R. australis* (lanes 2) were dissolved at room temperature (R) or heated (H) by boiling for 5 min. FISF (A) and QTT (B) convalescent-phase patient sera were reacted with each of these antigens.

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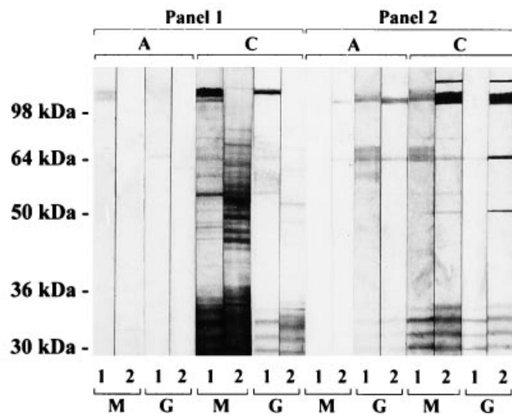

FIG. 4. SFG patient antibody responses to rickettsiae according to antibody class. IgM (M) and IgG (G) responses in acute-phase (A) and convalescent-phase (C) sera from FISF (panel 1) and QTT (panel 2) patients against *R. honei* (1) and *R. australis* (2) antigens are shown.

Rocky Mountain spotted fever and boutonneuse fever. The causative agents of these diseases, *Rickettsia rickettsii* and *Rickettsia conorii*, are extremely similar genetically. They have 99.7 and 99.8% homology between their 16S rRNA genes and 17-kDa-antigen genes, respectively. This homology is far closer than the genetic homology between *R. australis* and the FISF agent. In fact, according to the current information, *R. honei* is more closely related to these SFG members than to *R. australis* (1).

The failure to amplify the rOmp A gene product from the *R. australis* template by PCR further illustrates an important difference between this agent and other SFG members. It has been shown that the genetic characterization of the rOmp B gene implies that *R. australis* is the most evolutionarily distant rickettsia of the SFG (7, 8). This suggests that the phylogenetic position of *R. australis* needs to be reevaluated, and reclassification of *R. australis* away from the other SFG members may eventuate.

We thank Brian Dwyer and Robert Baird for their inspiring discussions and Josie Ramirez for expert secretarial assistance in the preparation of the manuscript.

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