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BARBASH STRAIN SPOTTED FEVER GROUP RICKETTSIA IS A STRAIN OF *RICKETTSIA CONORII* AND DIFFERS FROM *RICKETTSIA SIBIRICA*

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Summary. — The Barbash strain of spotted fever group rickettsia was reexamined in this study by the microimmunofluorescence test with mouse antisera and with monoclonal antibodies. Protein immunoblotting was performed for comparison of purified antigens of *R. rickettsii*, *R. sibirica*, *R. conorii* and Barbash strain. Comparison of Barbash strain, *R. rickettsii* (Sheila Smith strain), *R. conorii* (Malish 7 strain), and *R. sibirica* (strains 232, 246 and Jinghe-74) of the spotted fever group in the microimmunofluorescence test of Philip *et al.* revealed that Barbash strain has antigens that yield homologous titers with the *R. conorii* strains and differ from *R. sibirica* and *R. rickettsii*. Monoclonal antibodies specific for *R. conorii* react at identical titres with the Barbash strain, and a monoclonal antibody specific for *R. sibirica* does not react with the Barbash strain. Likewise, T-cell hybridomas reactive with *R. conorii* but not *R. sibirica* yield a strong response when stimulated by Barbash strain antigens.

Western immunoblotting with the same polyclonal and monoclonal antibodies confirmed the presence of specific protein antigens of *R. conorii* and different protein antigenic composition of *R. sibirica* when compared with Barbash strain. Thus, Barbash strain is a strain of *R. conorii*.

Key words: spotted fever group rickettsiae; serology; antigens

Introduction

Barbash strain has been distributed as a standard strain of *Rickettsia sibirica* by the World Health Organization (WHO). The Barbash strain was sent by the WHO to the People's Republic of China. Subsequently, some Chinese rickettsiologists have reported that several strains of spotted fever

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group (SFG) rickettsiae isolated in China were different from Barbash strain, *R. conorii*, *R. rickettsii*, *R. akari* and *R. parkeri*. Thus, this suggested that the Chinese strains of SFG rickettsiae represent new species (Kong *et al.*, 1985; Liu *et al.*, 1985; Wang *et al.*, 1984). In identifying the Chinese strains of SFG rickettsiae, we noticed that the Barbash strain did not appear to be a strain of *R. sibirica* and that the Chinese strains are *Rickettsia sibirica* (Wang, J. G., Lenz, B., and Walker, D. H., unpublished data). For this reason we examined the Barbash strain by microimmunofluorescence with mouse antisera prepared according to the protocol of Philip *et al.*, (1978) and with monoclonal antibodies and also by the Western immunoblotting method and by SFG rickettsial antigen-specific T-cell hybridomas.

Materials and Methods

Rickettsial strains. The strains that were used in this study and their backgrounds are listed in Table 1. Barbash strain (003 WHO EP5 5/13/1970) was sent to mainland China by the WHO. *R. conorii* (Malish 7 strain and Indian tick typhus strain) and *R. sibirica* (strain 246) were obtained from the American Type Culture Collection. *R. sibirica* (strain 232) was obtained from Mr. C. Pretzman (Ohio Department of Health Laboratory, Columbus, OH). *R. sibirica* (Jinghe-74) was provided by Dr. Y. F. Zhang (Chinese Academy of Preventive Medicine, Beijing). *R. rickettsii* (Sheila Smith strain) and Thai tick typhus rickettsia (TT-118 strain) were obtained from Dr. C. L. Wisseman, Jr. (University of Maryland, Baltimore, MD). The rickettsial passage histories comprise *R. conorii* (Malish 7 strain), chick embryo yolk sac passage (CE) 14, plaque-purified, *R. conorii* (Indian tick typhus strain), guinea pig passage (GP) 50+, CE 13, chick fibroblasts 1, plaque-purified, *R. sibirica* (strain 246), complete passage history unknown, CE 19, Vero cell passage (VC) 3, GP 1, *R. sibirica* (strain 232), complete passage history unknown, VC 8, *R. sibirica* (Jinghe-74), GP 1, CE 8, VC 2, *R. rickettsii* (Sheila Smith strain), GP 2, CE 12, chick fibroblasts 1, VC 3, plaque-purified, TT-118, complete passage history unknown, VC 3, plaque-purified, and Barbash strain, CE 10.

Antigens. Antigens for microimmunofluorescence (micro-IF) and for preparing mouse antisera were prepared in highly infected Vero cells. Vero cell monolayers in tissue culture flasks (150 cm^2) were inoculated with the rickettsial strains. On day 8–10 after inoculation, rickettsial

Table 1. Origins of the spotted fever group rickettsiae examined in this study

Rickettsial	Location	Host	Year
R. sibirica			
strain 232	U.S.S.R.	human	?
strain 246	U.S.S.R.	*Dermacentor nuttalli*	1949
Jinghe 74 strain	Xinjiang, China	*Dermacentor nuttalli*	1981
R. conorii			
Malish 7 strain	Witwatersrand, South Africa	human	1946
Indian tick typhus strain	India	*Rhipicephalus sanguineus*	1946
R. rickettsii			
Sheila Smith strain	Montana, USA	human	1946
Thai Tick Typhus			
TT-118	Thailand	mixed larval ticks	1962

growth was evaluated by indirect immunofluorescence (IFA), and rickettsiae were harvested and pelleted. The supernatant fluid was discarded and the pellet was resuspended either in 10 ml of sucrose-phosphate-glutamate buffer (SPG) (0.218 mol/l sucrose, 0.0038 mol/l KH_2PO_4, 0.0072 mol/l K_2HPO_4, 0.0049 mol/l monosodium L-glutamine acid, pH 7.0) for storage at −70 °C and subsequent mouse immunization or in 9 ml of 0.01 mol/l phosphate buffered saline (PBS), pH 7.4, for micro-IF slides. Microdots containing 0.025 ml were placed on 12-well slides and dried 30 min in a biohazard containment hood before fixation in absolute acetone for 10 min. Slides were stored at −70 °C until use.

Antigens for Western immunoblotting were cultivated in chicken-embryo yolk sac according to the method of Bell and Pickens (1953). Dilutions of rickettsial inocula were adjusted to cause embryo death around 5 days postinoculation. Yolk sacs were harvested after further incubation for one to two days. The rickettsiae were purified by Renografin density gradient centrifugation according to the method of Hanson *et al.* (1981) in a 30% − 36% − 42% discontinuous gradient. The light bands were washed in SPG and pelleted at 15,000 × g for 20 min. The pellets were resuspended in distilled water and stored at −70 °C.

Antisera. Antisera were prepared in male Swiss Webster mice according to the method of Philip *et al.* (1978). Stock antigens containing viable rickettsiae were diluted 1/10 in SPG, and 0.5 ml doses were injected into tail veins of 15 mice on days 0 and 7. Mice were exsanguinated on day 10. Sera from mice in each group were pooled to minimize host-variation in antibody responses, divided into 0.1 ml aliquots, and stored at −20 °C.

Monoclonal Antibodies. A six week old male Balb/c mouse (Charles River Laboratories, Wilmington, MA) was inoculated intraperitoneally on days 0 and 7 with 0.5 ml of a 10% yolk sac stock of *R. sibirica* (strain 246) diluted 1/5 in SPG.

Fusion was performed on day 10 using P3X63 − Ag8.653 myeloma cells and the aseptically harvested spleen cells (Oi and Herzenberg, 1980; Lange and Walker, 1984; Feng, *et al.* 1987). The cells were combined in the presence of 5% polyethylene glycol (MW 4,000, Sigma Chemical Co., St. Louis, MO) at a ratio of 1/4 (myeloma cells : spleen cells).

Hybridomas secreting monoclonal antibodies against *R. conorii* were prepared similarly. Two 8-week old male Balb/c mice were inoculated intraperitoneally with 0.3 ml of a 20% yolk sac suspension of *R. conorii* (Malish 7 strain) on days 0 and 17. Three days after the latter immunization spleen cells were collected and fused with P3X63-Ag8.653 myeloma cells at a ratio of spleen cells to myeloma cells of 10 : 1. The fused cells were suspended in a 1 : 1 mixture of Dulbecco's high glucose minimal essential medium and Ham's F-12 medium with 15 mmol/l HEPES buffer, 20 mmol/l L-glutamine, 14.3 mmol/l sodium bicarbonate, 15% foetal calf serum and 10^{-5} M 2-mercaptoethanol.

The cells were distributed into 96-well microtiter plates in the presence of selective media with approximately 3×10^5 spleen cells/well. After identification of antibody-secreting clones by IFA, the cells were transferred to flasks and then subcloned twice.

After the second subcloning, anti-*R. sibirica* hybridomas were transferred to 75 ml flasks, and the supernatant was collected for titration by IFA and for staining of Western immunoblots. The supernatant was frozen at −20 °C. After the second subcloning, anti-*R. conorii* hybridomas were inoculated into pristane-primed, irradiated female Balb/c mice for production of ascites fluid. The globulin fraction of the ascites fluid was purified by precipitation with a 50% saturated solution of ammonium sulphate and frozen at −70 °C until use.

Procedure for micro-immunofluorescence. After the 0.1 ml vials of antisera were thawed, the sera were absorbed for 20 min in a 37 °C waterbath with a 0.7 ml volume of PBS containing Vero cells from a confluent 20 cm^2 monolayer in order to minimize nonspecific immunofluorescent staining. Then sera were diluted in two fold increments from 1 : 8 to 1 : 16,384, and 0.025 ml of diluted serum was overlaid on each microdot. The slides were incubated at room temperature (23°) in a humid container for 30 min. Then the slides were washed in PBS and one drop of diluted rabbit antimouse immunoglobulin fluorescein isothiocyanate conjugate was added to each microdot for another 30 min incubation in the humid container. Then the slides were washed and mounted in buffered glycerol for reading.

The slides were coded and examined blindly on a Leitz Laborlux ultraviolet microscope equipped with barrier and exciter filters for fluorescein. The endopoint of the serum reaction was that one fourth of the quantity of rickettsiae present in the positive control microdots were identified clearly with the whole rickettsial outline visible at the highest titer. The code was broken after reading. The study was repeated.

Statistical methods. For determination of differences between two serotypes, the specificity difference (SPD) between each pair of strains was obtained by the method of Magnusson (1960) as modified by Fraser and Berman (1965) and as described by Philip *et al.* (1978):

$$SPD = (Aa + Bb) - (Ab + Ba),$$

where Aa is the antibody titer of serum A with the homologous antigen a, Bb is the homologous reaction of serum B with antigen b, Ab is the heterologous reaction of serum A with antigen b, and Ba is the heterologous reaction of serum B with antigen a. Units were substituted for actual antibody titers to give more convenient measures of strain relationships such that titres of 8 were designated as 1, titers of 16 = 2, 32 = 3, etc. Sera that were nonreactive at 1/8 dilution were considered to be devoid of antibody and were designated as 0. In the comparison of two strains that were antigenically related, the heterologous titres were similar to the homologous antibody titers, and the SPD was near 0. In the comparison of two strains that were antigenically dissimilar, heterologous titres were much lower than homologous titers, and the SPD was high. According to Philip *et al.* (1978) and Fraser *et al.* (1965), specimens with SPD < 3.0 are homologous serotypes, and SPD > 3.0 indicates heterologous strains ($p < 0.01$).

SDS-PAGE and Western immunoblotting. Sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli (1970) as modified by Dasch *et al.* (1985) to employ less harsh denaturing conditions. Stacking and separating gels consisted of 4 and 12.5% acrylamide, respectively. After the antigen stock were thawed and dissolved at 4 °C in sample buffer (0.0625 mol/l Tris base, pH 8, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, and 0.002% bromphenol blue), gel electrophoresis was performed at 20 mA/gel in a 4 °C cold room over 10 hr. Electrophoretically separated polypeptides were transferred from gels to 0.2 μm nitrocellulose paper (Sartorius) by electrophoresis at 195 mA for 2 hr followed by 100 mA for 2 hr as previously described (Hanff *et al.*, 1982; Towbin *et al.*, 1979), but in a 4 °C cold room. Antigens were detected on the nitrocellulose paper by immunoperoxidase staining after blocking nonspecific protein binding with 5% milk in 0.05 mmol/l Tris-HCl buffer, pH 7.5, for 1 hr at room temperature. Mouse sera were diluted in a solution containing 1% milk and 0.01 mol/l PBS, pH 7.5. The antisera, which generally had IFA titers of 1/2048, were diluted 1/150 except for the low titered antiserum to *R. rickettsii*, which was diluted 1/30. Culture supernatant fluid containing monoclonal antibody 5-2 was diluted 1 : 2 in the same solution. Purified ascites fluids containing monoclonal antibodies 61 and 24 were similarly diluted 1/200. After incubation with the primary antibody for 1 hr at room temperature, the nitrocellulose paper was washed in 0.01 mol/l PBS, pH 7.5, for 30 min. Then the papers were incubated with goat antimouse IgG diluted 1/300 in 1% milk, 0.01 mol/l PBS, pH 7.5, for 30 min and washed as before.

Yolk sac protein control was prepared from two yolk sacs harvested from 10 day old chicken embryos. Yolk sacs were triturated with a mortar and pestle and suspended in 20 ml of PBS The sespension was pelleted at 15,000 × g for 20 min. The supernatant fluid was discarded in 20 ml of PBS and washed two more times. Then the pellet was resuspended in 2 ml of distilled water and stored at −70 °C. The molecular weight standards (Bio-Rad Laboratories) were myosin, β-galactosidase, phosphorylase, bovine serum albumin, and ovalbumin. Their molecular weights are 200, 116, 92.5, 66, and 45 kD, respectively.

Production and testing of cloned T-cell hybridomas. Antigen-responsive T-cell hybridomas were prepared from immune splenic T-cells obtained from C3H/HeJ mice after immunization with *R. conorii* as previously described (Jarboe *et al.*, 1986). The cells used in the experiments described in this report have been cloned numerous times (more than 10) by limiting dilution. Responses to antigen were determined by production of interleukin-2 (IL-2) as assayed by proliferation of the IL-2-dependent cell line HT-2. Each data point represents the mean counts per minute of ^{3}H-thymidine uptake of triplicate HT-2 cultures in the presence of supernatant from antigen-stimulated hybridomas. Supernatant from hybridomas stimulated with medium alone serve as background controls.

Results

By the micro-IF assay, Barbash strain has a similar antibody titer as *R. conorii* (Malish 7 strain) and at least a fourfold difference in titre strains of *R. sibirica*, *R. rickettsii* and TT-118 (Tab. 2). The micro-IF titers

Table 2. Micro-immunofluorescence antibody titres from reciprocal cross-reactions of mouse antisera against R. rickettsii, R. conorii, and R. sibirica strains of spotted fever group rickettsiae

Mouse Antiserum against	Rickettsial Antigen						
	R. sib (232)	*R. sib* (246)	Jinghe-74 strain	Barbash strain	*R. con* (Malish 7)	*R. rick* (SS)	TT-118
R. sibirica							
strain 232	**512**[a]	**512**	**512**	64	64	32	32
strain 246	**256**	**256**	**256**	32	32	8	0
Jinghe-74	**512**	**512**	**512**	16	16	8	0
Barbash strain	16	32	16	**512**	**512**	16	32
R. conorii Malish 7 strain	64	64	64	**2048**	**2048**	64	32
R. rickettsii Sheila Smith strain	128	256	256	128	64	**2048**	128
TT-118	8	8	0	8	16	8	**1024**

[a] highest reactive reciprocal dilution of serum

Table 3. Mean specificity differences among homotypic strains and heterotypic strains of SFG rickettsiae

Against	Rickettsial Antigen						
	R. sib. (232)	*R. sib.* (246)	Jinghe-74	Barbash strain	*R. con.* (Malish 7)	*R. rick.* (SS)	TT-118
R. sibirica							
strain 232	**0**	**0**	**0**	8	8	8	11
strain 246	**0**	**0**	**0**	7	7	8	13
Jinghe-74	**0**	**0**	**0**	10	10	9	15
R. conorii							
Malish 7 strain	8	7	10	**0**	**0**	10	12
Barbash strain	8	7	10	**0**	**0**	9	11
R. rickettsii	8	8	9	9	10	**0**	11
TT-118	11	13	15	11	12	11	**0**

Table 4. Microimmunofluorescence titres selected monoclonal antibodies and SFG rickettsial antigens

Monoclonal antibody	Antigens of SFG rickettsiae							
	R. sibirica			*R. conorii*			*R. rickettsii*	TT-118
	232	246	Jinghe-74	Barbash strain	Malish 7	Indian tick typhus	Sheila Smith	
5-2	512[a]	512	512	—[b]	—	—	—	—
24	16	16	32	16,384	16,384	16,384	32	32
61	—	—	—	16,384	16,384	16,384	—	—

[a] reciprocal of highest dilution of serum yielding positive results

[b] negative at a dilution of 1 : 8

Table 5. Responses of cloned T-cell hybridomas to antigens of spotted fever group rickettsiae

Hybridoma	Media	*R. conorii*[a] (Malish 7 strain)	*R. rickettsii*[a] (Sheila Smith strain)	*R. conorii*[b] (Malish 7 strain)	Barbash strain[b]
32.13	675[c]	36,512	1,100	41,330	32,176
v.2	823	11,900	17,937	13,200	19,512

[a] — antigen prepared at Walter Reed Army Institute of Research
[b] antigen prepared at University of North Carolina
[c] each point represents mean counts/minute of triplicate cultures
Standard deviation was ≤15% and omitted

for the antigens of *R. sibirica* strains 232, 246 and Jinghe-74 are identical for each respective antiserum. *R. rickettsii* (Sheila Smith strain) and TT-118 are distinct from them. By the means of specificity differences, the SPD for *R. sibirica* strains 232, 246 and Jinghe-74 are 0, and compared with the other SFG rickettsial strains the SPD of the *R. sibirica* strains are 7 or greater (Tab. 3). The SPD for Barbash strain and *R. conorii* (Malish 7 strain) are 0, and the SPD for these strains are 7 or greater when compared with the other strains. The SPD of *R. rickettsii* and TT-118 when compared with each other strain are 8 or greater. Thus, Barbash strain and *R. conorii* (Malish 7 strain) have antigens that are homologous and differ from the *R. sibirica* strains, *R. rickettsii*, and TT-118. Barbash strain appears to be a strain of *R. conorii*.

The results of micro-IF with selected species-specific monoclonal antibodies are listed in Table 4. Monoclonal antibody 5-2 is specific for *R. sibirica*. Monoclonal antibody 61 is specific for *R. conorii*. Monoclonal antibody 24 reacts with *R. conorii* at a high titre and with the other SFG rickettsiae at a low titre. These results confirm that Barbash strain is a strain of *R. conorii*.

Cloned T-cell hybridomas reactive with *R. conorii* only (clone 32.12) and crossreactive only with *R. conorii* and *R. rickettsii* (clone V.2) were reacted with purified whole rickettsia antigens of *R. conorii* (Malish 7 strain) and *R. rickettsii* (Sheila Smith strain) prepared at Walter Reed Army Institute of Research and *R. conorii* (Malish 7 strain) and Barbash strain prepared at the University of North Carolina. As can be seen from this experiment, the antigen prepared from Barbash strain stimulated IL-2 production by the T-cell hybridoma that was shown previously and in this study to be responsive only to an epitope specific for *R. conorii* (Tab. 5). The Barbash antigen also contained epitopes common to *R. conorii* and *R. rickettsii* as evidenced by the ability to stimulate the T-cell hybridoma (V.2) responsive to these two rickettsiae. These data would suggest that the Barbash strain used in these experiments contained epitopes recognized by the antigen receptor on cloned T-cell hybridomas that are unique to strains of *R. conorii*.

Immunoblotting results obtained with the same immune sera from mice used in the micro-IF assay and with monoclonal antibodies for *R. sibirica* and *R. conorii* and their reactions against the antigens of *R. rickettsii* (Sheila Smith strain), *R. sibirica* (strain 232), Barbash strain, *R. conorii* (Malish 7 strain), and yolk sac control are shown in Figures 1—6. Fig. 1 demonstrates the rickettsial antigens that are reactive with mouse anti-*R. sibirica* (strain 232) immune serum. It shows that both Barbash strain and *R. conorii* (Malish 7 strain) have two antigenic bands of the same molecular weights (139 kD and 115 kD). *R. sibirica* (strain 232) has bands (145, 131, 117, and 112 kD) that differ from the Barbash strain, and the antigenic bands of *R. rickettsii* also demonstrate a different pattern (195, 149, 132, and 115 kD).

Fig. 2 demonstrates the rickettsial antigens that are reactive with mouse anti-*R. conorii* (Malish 7 strain) immune serum. It demonstrates similar antigenic bands as figure 1; however, *R. conorii* has additional bands of 163 kD and 112 kD. The Barbash strain also has the band of 163 kD which appears somewhat less quantitatively on the basis of the amount of protein antigen loaded into the well.

Fig. 3 shows the reaction of the SFG rickettsial antigens with mouse anti-Barbash strain immune serum. Adjustment of the quantity of Barbash strain and *R. conorii* (Malish 7 strain) both contain major bands of 163, 139, and 115 kD. In contrast, anti-Barbash strain serum demonstrates two major antigenic proteins of *R. sibirica* (strain 232) with distinct molecular sizes (117 and 112 kD) and a distinct 132 kD major antigenic protein of *R. rickettsii*.

Reaction of the same antigens with monoclonal antibody 5-2, which is specific for strains of *R. sibirica*, demonstrates one strong 131 kD band for the proteins of only *R. sibirica* (Fig. 4). Immunoblotting with monoclonal antibody 61, which reacts specifically with strains of *R. conorii*, demonstrated that only the Barbash strain and *R. conorii* (Malish 7 strain) have a major antigenic band of 139 kD and a broad band (155—182 kD) (Fig. 5). Immunoblot assay with monoclonal antibody 24, which reacts as a high titre with strains of *R. conorii* and at a low titre with the other SFG rickettsiae yielded similar results (Fig. 6). These immunoblotting results further confirm that Barbash strain is a strain of *R. conorii* and not *R. sibirica*.

Discussion

This investigation accomplished the antigenic reidentification of the Barbash strain as *R. conorii* rather than *R. sibirica*. This antigenic analysis of the antigens of Barbash strain and comparison with other established SFG rickettsial strains employed an array of recently developed methods. The composite results of microimmunofluorescence, monoclonal antibody reactivity, T-cell clone reactivity, and Western immunoblotting examined not only species-specific epitopes but also examined antigens with crossreactive epitopes with polyclonal antisera. The conclusion is, therefore, quite well supported that the species designation of the Barbash strain is *R. conorii*.

This reassignment of species classification has importance for previous studies in which the Barbash strain has been used as a prototype strain of *R. sibirica*. Particularly those studies, which have proposed new rickettsial species of the SFG such as *R. sinkiangensis*, *R. heilongjiangi*, and *R. slovaca*, should be reexamined with a different standard strain of *R. sibirica* (Kong *et al.*, 1985; Wang *et al.*, 1984; Lou *et al.*, 1985; Úrvölgyi and Brezina, 1978).

The microimmunofluorescence test in this study yielded two other noteworthy results. The designation of the Thai tick typhus rickettsia, TT-118, as a distinct SFG rickettsial species on the basis of mouse toxicity neutralization testing was confireed by the micro-IF test (Robertson and Wisseman, 1973). Moreover, the SFG rickettsial strain Jinghe-74 (Wang *et al.*-1984) was demonstrated by micro-IF to be a strain of *R. sibirica* indistinguishable from strains 246 and 232.

It is anticipated that further application of the technology of monoclonal antibodies, Western immunoblotting, and cloned T-cell hybridomas will achieve a better understanding of the antigenic composition of the members of the SFG of rickettsiae. The applications of specific antigen identification and detection to epidemiology, diagnosis, and vaccine development may be expected to produce much needed progress in rickettsiology in the near future.

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Legends to Figures (Plates LXX–LXXV):

Fig. 1. Immunoblot assay with mouse antiserum against *R. sibirica* (strain 232). Antigens are *R. rickettsii* (lane 1), *R. sibirica* (lane 2), Barbash strain (lane 3), *R. conori* (Malish 7 strain) (lane 4), and normal yolk sac (lane 5). The molecular weight standards from 200 kD to 45 kD are designated by the arrows.

Fig. 2. Immunoblot assay with mouse antiserum against *R. conorii* (Malish 7 strain). The antigens are *R. rickettsii* (lane 1), *R. sibirica* (lane 2), Barbash strain (lane 3), *R. conorii* (Malish 7 strain) (lane 4), and normal yolk sac (lane 5). The molecular weight standards (arrows) are from 200 kD to 45 kD.

Fig. 3. Immunoblot assay with mouse antiserum against Barbash strain SFG rickettsia. The antigens are *R. rickettsii* (lane 1), *R. sibirica* (lane 2), Barbash strain (lane 3), *R. conorii* (Malish 7 strain) (lane 4), and normal yolk sac (lane 5). The molecular weight standards (arrows) are from 200 kD to 45 kD.

Fig. 4. Immunoblot assay with monoclonal antibody 5-2 which reacts specifically with stains of *R. sibirica*. The antigens are *R. rickettsii* (lane 1), *R. sibirica* (lane 2), Barbash strain (lane 3), *R. conorii* (Malish 7 strain) (lane 4), and normal yolk sac (lane 5). The molecular weight standards (arrows) are 200 kD, 116 kD, 92 kD, 66 kD, and 45 kD.

Fig. 5. Immunoblot assay with monoclonal antibody 61, which react specifically with strains of *R. conorii*. The antigens are *R. rickettsii* (lane 1), *R. sibirica* (lane 2), Barbash strain (lane 3), *R. conorii* (Malish 7 strain) (lane 4), and normal yolk sac (lane 5). The molecular weight standards (arrows) are from 200 to 45 kD.

Fig. 6. Immunoblot assay with monoclonal antibody 24 which reacts at a high titre with *R. conorii* and at lower titres with other SFG rickettsiae. The antigens are *R. rickettsii* (lane 1), *R. sibirica* (lane 2), Barbash strain (lane 3), *R. conorii* (Malish 7 strain) (lane 4), and normal yolk sac (lane 5). The molecular weight standards (arrows) are from 200 to 45 kD.