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Protective Monoclonal Antibodies Recognize Heat-Labile Epitopes on Surface Proteins of Spotted Fever Group Rickettsiae

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Thirty-eight monoclonal antibodies that have not been reported previously were developed from mice immunized with *Rickettsia rickettsii*, *R. conorii*, and *R. sibirica*. Western immunoblotting showed that these monoclonal antibodies are directed against heat-sensitive epitopes which are located on two major surface polypeptides with molecular sizes ranging from 115 to 150 kilodaltons. The detection of the two bands did not depend on the presence of 2-mercaptoethanol. Both bands were destroyed by treatment with proteinase K. Monoclonal antibodies examined by immunofluorescence assay reacted with epitopes that are species specific, group reactive, or shared among a smaller subset of species of spotted fever group rickettsiae. Nine of the monoclonal antibodies were evaluated for their ability to neutralize rickettsial infection and thus protect animals against disease caused by homologous species of rickettsiae. Treatment of rickettsiae with monoclonal antibodies F3-12, F3-14, and F3-36 completely protected guinea pigs against illness caused by the homologous organism *R. rickettsii*. Monoclonal antibodies F9-5G11 and F15-5B12, derived from mice immunized with *R. sibirica*, conferred partial protection by delaying the onset and shortening the duration of fever in guinea pigs inoculated with *R. sibirica*. Monoclonal antibodies F2-15, F2-31, F2-53, and F3-12 protected mice from a lethal infection with *R. conorii*. Heat-labile epitopes of spotted fever group rickettsial surface proteins are important candidate antigens for development of vaccines to confer protective immunity.

Rickettsiae are obligate intracellular bacteria which are closely associated with ticks, mites, lice, fleas, and chiggers. The spotted fever group (SFG) of the genus *Rickettsia* comprises numerous antigenically related species, including the etiologic agents of geographically widespread human diseases, i.e., *Rickettsia rickettsii* (Rocky Mountain spotted fever), *R. conorii* (boutonneuse fever), *R. sibirica* (North Asian tick typhus), *R. australis* (Queensland tick typhus), and *R. akari* (rickettsialpox).

Monoclonal antibodies (MAbs) to rickettsial antigens are powerful tools for identifying which antigenic epitopes are immunodominant and provide a means of determining the physical location of these epitopes. MAbs also provide a mechanism with which to identify rickettsial isolates. Several studies have indicated that the majority of MAbs derived from mice inoculated with SFG rickettsiae react with either heat-sensitive or heat-resistant epitopes of the 128- to 155-kilodalton (kDa) and 110- to 133-kDa polypeptides (3, 4, 11). Pretreatment of rickettsiae with polyclonal antibodies to a surface protein or with most of the MAbs to the heat-sensitive epitopes confers protection against febrile infection of guinea pigs, protection against infectious death of susceptible mice, and prevention of the mouse toxicity phenomenon (1, 4, 5, 11, 16). In contrast MAbs to the heat-resistant protein epitopes and MAbs reactive with the proteinase K-resistant lipopolysaccharide (LPS)-like surface antigens fail to confer protection against rickettsial infection or the mouse toxicity phenomenon. Some MAbs to heat-sensitive and heat-resistant protein antigens have been shown to cross-react with antigens represented among most SFG rickettsial species but not *R. akari* and *R. australis*. MAbs reactive with the LPS-like antigens cross-react with all rickettsiae of the SFG and none of the typhus group rickettsiae tested (3). It seems likely that the LPS-like components are the group-specific antigens of the SFG rickettsiae. The present study was undertaken to determine whether SFG rickettsiae have species-specific and group-reactive protein epitopes and, if so, to analyze the physical and immunologic characteristics of the epitopes. In this study, we developed species-specific MAbs for *R. conorii* and *R. rickettsii*, a MAb that distinguished *R. sibirica* from other SFG rickettsiae at selected dilutions, and MAbs that reacted with protein antigens of all SFG rickettsiae examined including *R. akari* and *R. australis*. Both species-specific and group-reactive MAbs directed against the heat-sensitive epitopes of surface proteins of *R. rickettsii* (135 and 150 kDa) and *R. sibirica* (115 and 135 kDa) protected guinea pigs against the homologous rickettsiae. Furthermore, species-specific and group-reactive MAbs for the heat-sensitive epitopes of surface proteins of *R. conorii* (115 and 135 kDa) protected susceptible mice from morbidity and mortality of *R. conorii*.

MATERIALS AND METHODS

Rickettsiae. *R. rickettsii* Sheila Smith was obtained from C. L. Wisseman, Jr. (University of Maryland, Baltimore). *R. conorii* Malish 7, *R. sibirica* 246, and *R. akari* MK were obtained from the American Type Culture Collection. *R. sibirica* 232, *R. australis* W58, and Thai tick typhus rickettsia TT-118 were obtained from C. Pretzman (Ohio Department of Health Laboratory, Columbus. *R. sibirica* An-84 was isolated from a patient in China (10). The passage history of these rickettsial strains has been described recently (20). All rickettsial stocks were plaque-purified and mycoplasma free. Infectious yolk sac stocks of *R. conorii*, *R. rickettsii*, and *R. sibirica* were diluted in brain heart infusion broth as 10% suspensions and were stored at −70°C in 1-ml aliquots. Rickettsiae were cultivated in yolk sacs of 5-day-old specific-pathogen-free embryonated hen eggs (SPAFAS, Inc., Norwich, Conn.). Infectious yolk sacs were harvested, and rickettsial organisms were purified by centrifugation in diatrizoate meglumine (Berlex Laboratories,

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Inc., Wayne, N.J.) discontinuous density gradients as described previously (14).

Animals. Female BALB/c mice (6 to 8 weeks of age) and male C3H/HeJ mice (8 weeks of age) were purchased from Jackson Laboratory, Bar Harbor, Maine. Male guinea pigs weighing 500 g each were purchased from Charles River Breeding Laboratories, Inc., Wilmington, Mass.

Development of MAbs. Two 8-week-old female BALB/c mice were each inoculated intraperitoneally with 2×10^4 PFU of an infectious yolk sac suspension of viable *R. rickettsii* on days 0 and 32, and two similar mice were each inoculated with $6.6 \times 10^{5.25}$ PFU of *R. conorii* on days 0 and 16. Three days after the latter immunization, spleen cells were collected and fused with P3X63-Ag-8.653 myeloma cells by using 50% polyethylene glycol (M_w, 3,500; Sigma Chemical Co., St. Louis, Mo.) at a ratio of spleen cells to myeloma cells of 10:1 as described previously (16). The cells were distributed into 96-well microdilution plates at 2×10^5 spleen cells per well in selective media. Anti-SFG-rickettsial-antibody-secreting cultures were detected by indirect immunofluorescence assay (IFA) with selection of antibodies that yielded the distinct rickettsial outline previously associated with reactivity with surface proteins (11). MAbs showing reactivity by IFA characteristic of anti-LPS antibodies as previously described (11) were excluded from further studies. The cells were transferred to flasks and then cloned twice by limiting dilution. After the second cloning, the antibody-producing hybridomas were inoculated into pristane-primed, irradiated female BALB/c mice for production of ascites fluid. The globulin fraction of the ascites fluid was precipitated with a 50% saturated solution of ammonium sulfate extensively dialyzed against saline and frozen at −70°C in 1- or 0.1-ml portions. Hybridomas secreting MAbs against *R. sibirica* (strains 246 and An-84) were prepared similarly, as described previously (11). MAb against Vero cell cytoskeletal protein had been prepared previously (11) in our laboratory.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed by the method of Laemmli (15). Density gradient-purified rickettsial-antigen stocks were thawed, dissolved, and examined in one of four ways: (i) addition of sample buffer (0.625 M Tris base [pH 8], 2% sodium dodecyl sulfate, 5% 2-mercaptoethanol [2-ME], 10% glycerol, and 0.002% bromophenol blue) and electrophoretic separation in 12.5% polyacrylamide gels at 20 mA per gel in a 4°C cold room for 16 to 18 h (11); (ii) addition of sample buffer, heating at 100°C for 5 min, and gel electrophoresis at room temperature for 12 to 14 h; (iii) treatment of antigens with proteinase K (type XI; Sigma) at 28 U/ml of sample at 37°C for 1.5 h prior to addition of final sample buffer and gel electrophoresis at 4°C for 18 h; or (iv) dissolution of antigen in sample buffer with or without 2-ME for different periods (1 h, 30 min, or 5 min) at room temperature and gel electrophoresis at 4°C for 18 h. Electrophoretically separated polypeptides were transferred from gels to 0.2-μm-pore-size nitrocellulose paper (Sartorius, Hayward, Calif.) by electrophoresis at 195 mA in buffer solution containing 2.5 mM Tris base, 192 mM glycine, and 20% methanol in a 4°C cold room for 4 h or at room temperature for 2 to 3 h. Antigens were detected on the nitrocellulose paper after nonspecific protein binding was blocked with 5% nonfat dry milk in phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4 [pH 7.2]) for 1 h at room temperature by reaction with MAbs diluted 1:100 in 1% milk in PBS. After being incubated with the primary antibody for 30 min at room temperature, the nitrocellulose sheet was washed for 20 min in PBS containing 0.05% Tween 20. The nitrocellulose sheet was then incubated for 30 min with goat anti-mouse immunoglobulin G–horseradish peroxidase conjugate (Bio-Rad Laboratories, Richmond, Calif.) diluted 1:500 in 1% milk in PBS, washed as before, and reacted with 1 mM color reagent (3,3′-diaminobenzidine tetrahydrochloride; Eastman Kodak Co., Rochester, N.Y.) and 0.04% hydrogen peroxide. The MAb against the Vero cell cytoskeleton and polyclonal murine anti-*R. rickettsii* and anti-*R. conorii* immune sera were used as controls.

Protection of animals. The median febrile doses of *R. rickettsii* and *R. sibirica* 232 were determined for guinea pigs by intraperitoneal inoculation of serial dilutions of stocks that were allowed to incubate at room temperature for 1 h prior to inoculation. MAbs against *R. rickettsii* and *R. sibirica* and normal mouse sera were diluted 1:10 in sucrose-phosphate-glutamate buffer, pH 7.0 (0.218 M sucrose, 0.0038 M KH_2PO_4, 0.0086 M Na_2HPO_4, 0.0049 M L-glutamic acid). Ten median febrile doses of *R. rickettsii* and *R. sibirica* were prepared by dilution of the 10% infectious yolk sac stocks 1:500 (*R. rickettsii*) and 1:50 (*R. sibirica*) in a solution containing 10% MAb or normal mouse serum, and then the mixture was incubated at room temperature for 1 h. A 2-ml volume of the suspension of rickettsiae and antibody was inoculated intraperitoneally into each guinea pig. The body temperature of guinea pigs prior to inoculation was less than 39.5°C. Guinea pigs were examined daily, and rectal temperatures were taken. Each group of animals inoculated with one of the antibody-rickettsia suspensions contained three guinea pigs.

The median lethal dose of *R. conorii* Malish 7 was determined for C3H/HeJ mice. Mice inoculated with a 10^{-9} dilution of stock rickettsiae died. Mice inoculated with a 10^{-10} dilution developed specific antibodies to *R. conorii* but did not die. Ten median lethal doses of *R. conorii* were mixed with MAb and incubated at room temperature for 40 min before being inoculated into mice. MAbs which were selected for animal protection were F2-15 (species specific for *R. conorii* and reactive with the 115-kDa polypeptide), F2-31 (species specific for *R. conorii* and reactive with the 135-kDa polypeptide), F2-53 (reactive with all SFG rickettsiae tested and the 135-kDa polypeptide of *R. conorii*), F3-12 (derived from mice immunized with *R. rickettsii* and reactive with *R. conorii* by IFA and immunoblotting), and F3-47 (derived from mice immunized with *R. rickettsii*; reactive with *R. conorii* by IFA but nonreactive by immunoblotting). Diluted normal mouse serum was reacted with *R. conorii* as a control. MAb F2-15 was diluted 1:5 in PBS, MAb F3-12 was diluted 1:10, and MAbs F2-31, F2-53, and F3-47 and normal mouse serum were diluted 1:20. Mice were observed for illness and death for 15 days after inoculation.

Colloidal gold-labeled protein A ultrastructural demonstration of presence and location of reactivity of MAbs with SFG rickettsiae. Density gradient-purified rickettsiae were diluted 1:6 in PBS, and 10 μl of the rickettsial suspension was placed on each 200-mesh grid coated with Formvar and allowed to adsorb for 10 min at room temperature. MAbs were diluted 1:100 in PBS, and 10 μl of the diluted MAb was placed on each grid and incubated for 30 min. The grids were washed five times with PBS. Colloidal gold (15 nm)-labeled protein A (Janssen Life Sciences Products, West Chester, Pa.) was diluted 1:10 in PBS; 10 μl was added to each grid and incubated for 30 min. The grids were washed as described above, and excess liquid was absorbed with filter paper.

TABLE 1. Reactivity of MAbs from mice immunized with *R. conorii* with various species of SFG rickettsiae

MAb[a]	Titer[b] for:				
	R. rickettsii	*R. conorii*	*R. sibirica*	*R. akari*	*Rickettsia* strain TT-118
F2-1	10,240	81,960	320	81,920	—
F2-5	40,960	40,960	40,960	40,960	—
F2-7	5,120	10,240	2,560	10,240	—
F2-8	—	1,280	640	320	5,120
F2-10	—	40,960	—	—	—
F2-15	—	10,240	—	—	—
F2-16	40,960	40,960	320	640	—
F2-18	2,560	5,120	2,560	2,560	2,560
F2-20	—	20,480	—	—	—
F2-24	80	20,480	640	—	—
F2-30	80	81,920	81,920	—	40,960
F2-31	—	81,920	—	—	—
F2-34	—	81,920	—	—	160
F2-45	320	2,560	1,280	640	2,560
F2-46	10,240	20,480	—	—	—
F2-48	—	40,960	—	—	40
F2-53	320	81,920	81,920	81,920	81,920
F2-57	160	81,920	80	80	2,560
F2-60	81,920	10,240	2,560	1,280	320
F2-60-2	10,240	20,480	—	—	—
F2-61	—	40,960	—	—	—
F2-64	10,240	20,480	5,120	40	5,120
F2-70	—	2,560	—	—	—

[a] MAbs F2-5, F2-18, F2-45, F2-53, F2-57, F2-60, and F2-64 were tested by IFA and found to be reactive with *R. australis*. In Western immunoblotting under native conditions, all MAbs reacted with *R. conorii* 135-kDa antigen, except for F2-15, which reacted with a 115-kDa antigen.

[b] Reciprocal titer by IFA against the indicated rickettsial species. —, Negative result.

TABLE 2. Reactivity of MAbs from mice immunized with *R. rickettsii* or *R. sibirica* with various species of SFG rickettsiae and with antigens of homologous species

MAb[a]	Titer[b] for:					
	R. rickettsii	*R. conorii*	*R. sibirica*	*R. akari*	*Rickettsia* strain TT-118	Antigen size (kDa)[c]
F3-5	40,960	—	40,960	—	10,240	150
F3-12	40,960	20,480	40,960	—	40,960	150
F3-14	163,840	—	1,280	—	—	150
F3-18	81,920	—	—	—	—	150
F3-24	81,920	—	—	—	—	135
F3-27-1	40,960	40,960	—	40,960	—	150
F3-28	20	—	—	—	—	135
F3-30	163,840	—	—	—	—	135
F3-34	20,480	—	—	—	—	135
F3-36	10,240	—	5,120	—	20,480	135
F3-39	40,960	320	320	80	160	135
F3-47	81,920	40,960	20,480	40,960	5,120	150
F3-57	5,120	2,560	640	5,120	—	135
F9-5G11	160	—	5,012	—	—	130
F15-5B12	524,288	—	524,288	—	40,960	115

[a] MAbs with prefix F3 were derived from mice immunized with *R. rickettsii*. MAbs F9-5G11 and F15-5B12 were derived from mice immunized with *R. sibirica*. MAbs F3-39 and F3-47 were tested by IFA and found to be reactive with *R. australis*.

[b] Reciprocal titer by IFA against the indicated rickettsial species. —, Negative result.

[c] Molecular size of the antigen of the homologous species with which the MAb reacts in Western immunoblotting under native conditions.

Control sera included normal mouse serum and polyclonal immune sera. The grids were examined with a Philips 201 electron microscope for the presence and location of the electron-dense colloidal gold particles (11).

Isoelectric focusing and IFA titrations. Isoelectric focusing and IFA titrations against antigens of *R. rickettsii*, *R. conorii*, *R. sibirica*, *R. akari*, Thai tick typhus rickettsia TT-118, and *R. australis* were performed as described previously (13, 18).

RESULTS

Thirty-eight new MAbs reactive with antigens of SFG rickettsiae were developed. Twenty-three MAbs were derived from BALB/c mice immunized with *R. conorii* (Table 1), thirteen were derived from mice immunized with *R. rickettsii* (Table 2), and two were derived from mice immunized with *R. sibirica* (Table 2). Isoelectric focusing demonstrated that after the second subcloning, all 38 antibodies secreted by the hybridomas were monoclonal.

The IFA titers of the MAbs against *R. rickettsii*, *R. conorii*, *R. sibirica*, *R. akari*, and strain TT-118 are presented in Table 1, along with the molecular sizes of the reactive polypeptides of the homologous rickettsiae. All of the MAbs reacted with heat-labile epitopes, and all except F3-47 reacted with surface antigens as determined by immunoelectron microscopy. Twenty-three MAbs, designated by the prefix F2, were derived from BALB/c mice immunized with *R. conorii*. All of them reacted with heat-labile epitopes of the 135-kDa surface protein except for F2-15, which reacted with the 115-kDa surface protein (Fig. 1). Six MAbs were species specific for *R. conorii*; six MAbs were reactive with all SFG rickettsiae examined, including *R. australis*; and the rest of the MAbs reacted with two to four of the SFG rickettsial species examined. Thirteen MAbs, designated by the prefix F3, were derived from BALB/c mice immunized with *R. rickettsii*. These MAbs reacted most strongly with

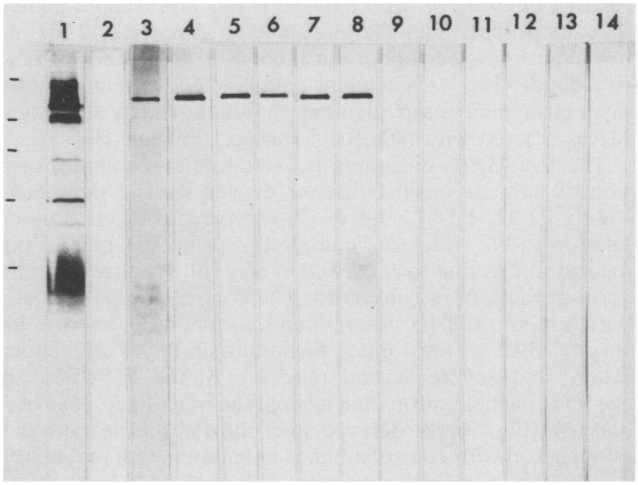

FIG. 1. Immunoblots of native antigens (lanes 1 to 8) and heat-denatured antigens (lanes 9 to 14) of *R. conorii* reacted with polyclonal mouse anti-*R. conorii* serum (lane 1), MAb against Vero cell cytoskeleton (lane 2), and anti-*R. conorii* MAbs F2-10 (lanes 3 and 9), F2-30 (lanes 4 and 10), F2-31 (lanes 5 and 11), F2-53 (lanes 6 and 12), F2-57 (lanes 7 and 13), and F2-61 (lanes 8 and 14). The molecular size standards are 200, 116, 97, 66, and 42 kDa (top to bottom, respectively, on the left).

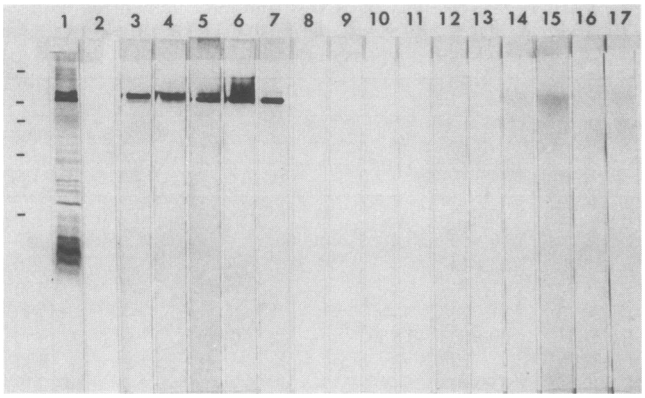

FIG. 2. Immunoblots of native antigens (lanes 1 to 7), heat-denatured antigens (lanes 8 to 12), and proteinase K-treated antigens (lanes 13 to 17) of *R. rickettsii* reacted with polyclonal mouse anti-*R. rickettsii* serum (lane 1), MAb against Vero cell cytoskeleton (lane 2), and anti-*R. rickettsii* MAbs F3-12 (lanes 3, 8, and 13), F3-14 (lanes 4, 9, and 14), F3-30 (lanes 5, 10, and 15), F3-36 (lanes 6, 11, and 16), and F3-39 (lanes 7, 12, and 17). The molecular size standards are 200, 116, 97, 66, and 42 kDa (top to bottom, respectively, on the left).

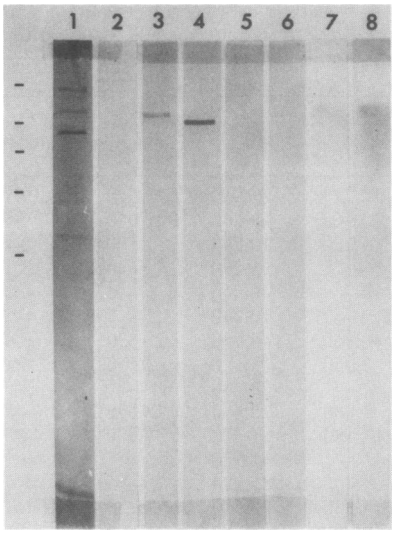

FIG. 3. Immunoblots of native antigens (lanes 1 to 4), heat-denatured antigens (lanes 5 to 6), and proteinase K-treated antigens (lanes 7 to 8) of *R. sibirica* reacted with polyclonal mouse anti-*R. sibirica* serum (lane 1), MAb against Vero cell cytoskeleton (lane 2), and anti-*R. sibirica* MAbs F9-5G11 (lanes 3, 5, and 7) and F15-5B12 (lanes 4, 6, and 8). The molecular size standards are 200, 116, 97, 66, and 42 kDa (top to bottom, respectively, on the left).

either the 135- or 150-kDa heat-labile protein antigen, although some MAbs did react with both polypeptides (Fig. 2). Five of the F3 MAbs reacted with species-specific epitopes of *R. rickettsii*, two of them reacted with all species of the SFG tested including *R. australis* (data not shown), and the rest of them reacted with two to four members of the five SFG rickettsiae examined. MAb F3-28 reacted poorly with acetone-fixed rickettsial antigen by IFA; however, it showed a strongly reactive pattern by Western blotting and immunoelectron microscopy. MAbs developed from mice immunized with *R. sibirica* (F9-5G11 and F15-5B12) reacted with heat-labile epitopes of the 115- or 130-kDa protein of *R. sibirica* (Fig. 3).

Heat-labile epitopes are shared between the two surface polypeptides of SFG rickettsiae (Fig. 4). However, the relative quantity of each epitope is not represented equally on each polypeptide. The presence or absence of 2-ME did not affect the electrophoretic mobilities or number of reactive bands (Fig. 4). Colloidal gold-labeled protein A immunoelectron microscopy demonstrated that nearly all of these MAbs reacted with rickettsial surface antigens (Fig. 5).

The five MAbs evaluated provided demonstrable protection of animals against disease caused by the rickettsiae. MAbs F3-12, F3-14, and F3-36, derived from mice inoculated with *R. rickettsii*, completely protected guinea pigs against illness due to *R. rickettsii* (Fig. 6). Neither fever nor scrotal reaction was observed. The control group inoculated with a mixture of *R. rickettsii* and normal mouse serum had fever (>39.5°C) for 4 days, beginning on day 5 after inoculation, and severe scrotal reaction. MAbs F15-5B12 and F9-5G11 partially protected against the inoculated dose of *R. sibirica* (Fig. 7). A delayed and shorter febrile period in comparison with control animals inoculated with the mixture of *R. sibirica* and normal serum was observed. Fever began on day 5 or 6 after inoculation of MAb-exposed *R. sibirica* and lasted for 1 to 2 days. In contrast, fever began on day 4 and lasted 3 to 5 days in the control group inoculated with *R. sibirica* exposed to normal serum. Comparison of the sums of the differences in body temperature on days 3 to 7 of animals inoculated with the MAb-treated rickettsiae and of the control serum-treated rickettsiae demonstrated that there was a significantly lower febrile response in the guinea pigs protected from *R. sibirica* by F15-5B12 ($P < 0.01$) and F9-5G11 ($P < 0.05$) and protected from *R. rickettsii* by each of the MAbs tested ($P < 0.01$) by Student's *t* test. Scrotal reactions were distinctly milder in the MAb-exposed group. All of the tested MAbs that were derived from mice immunized with *R. conorii* conferred complete protection on C3H/HeJ mice against challenge with 10 median lethal doses of *R. conorii*. Of the group-reactive MAbs reacted with *R. conorii* before challenge, only the MAb showing reactivity with *R. conorii* by Western immunoblotting conferred protection; the group-reactive MAb that did not show reactivity with *R. conorii* by Western immunoblotting or immunoelectron mi-

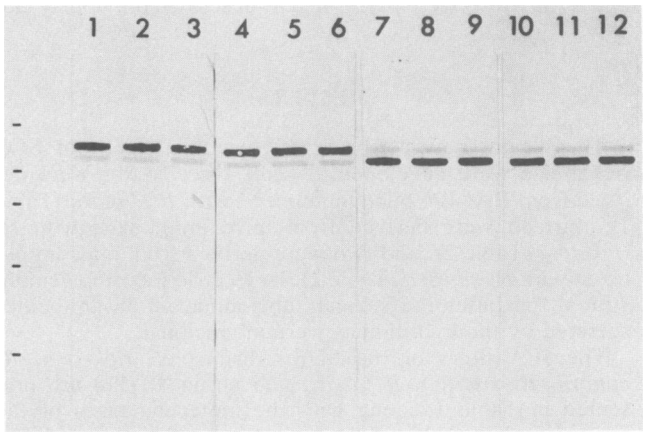

FIG. 4. Immunoblots of antigens of *R. rickettsii* dissolved in sample buffer with 5% 2-ME (lanes 1 to 3 and 7 to 9) and without 2-ME (lanes 4 to 6 and 10 to 12) for 1 h (lanes 1, 4, 7, and 10), 30 min (lanes 2, 5, 8, and 11), or 5 min (lanes 3, 6, 9, and 12) and reacted with MAbs F3-18 (lanes 1 to 6) and F3-30 (lanes 7 to 12). The molecular size standards are 200, 116, 97, 66, and 42 kDa (top to bottom, respectively, on the left).

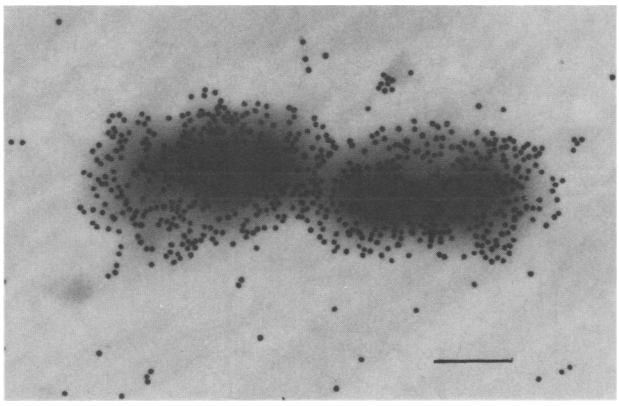

FIG. 5. Electron micrograph of *R. conorii* coated with colloidal gold-labeled protein A reacted with MAb F2-45, demonstrating the presence of the reactive epitope of this protein on the surface of the rickettsiae. Bar = 240 nm.

croscopy did not confer cross-protection against *R. conorii*. The mice in the control group showed 100% morbidity and 80% mortality after challenge with *R. conorii* (Table 3).

DISCUSSION

The importance of heat-labile surface antigens of rickettsiae to immunity was noted more than 40 years ago. Heat altered the immunological properties of rickettsial antigens of both *R. typhi* and *R. prowazekii* such that the heated antigens were indistinguishable (19). Fulton and Begg hypothesized that the rickettsiae have essentially identical surface structures and that the native heat-labile component has both species-specific and common antigenic determinants (12). Craigie et al. (6) proposed that the different antigenic determinants of rickettsiae are due to separable molecular entities. Dasch et al. extracted species-specific antigens from *R. typhi* and *R. prowazekii* (7, 9) and demonstrated several of their important properties, including heat lability and ability to stimulate protection of animals from infection (8). The molecular structure of these species-specific antigens and their significance in pathogenesis and protective immunity are unclear.

Anacker et al. reported that MAbs against LPS-like antigens of SFG rickettsiae are group specific and implied that these MAbs could be used to identify all members of the SFG (3). They found that many MAbs against *R. rickettsii* react with epitopes on proteins of *R. conorii*, *R. sibirica*, *R. parkeri*, and *R. montana* but do not react with *R. akari* or *R. australis*. We have developed a number of previously unreported MAbs to species-specific epitopes and other MAbs to broad-group-reactive epitopes of SFG rickettsial proteins. The MAbs were directed against heat-labile protein antigens which are located on the rickettsial surface. Most of the MAbs derived from BALB/c mice immunized with *R. conorii* were directed against the 135-kDa heat-sensitive surface protein. Each polypeptide that contained the species-specific or group-reactive epitopes had the same electrophoretic mobility; in other words, those different epitopes appear to reside on the same polypeptide.

The presence or absence of 2-ME in sample buffer used for dissolution of the antigens of *R. rickettsii* did not affect the electrophoretic mobility of the two major bands or their ability to bind specific MAbs. These observations suggest that the smaller polypeptide band is not derived from the other band by reduction of disulfide bonds. The observation that some MAbs react with the two bands with different intensities demonstrates that the two major bands share some epitopes. Epitopes that confer species specificity and group reactivity are located on the 135- and 150-kDa polypeptides of *R. rickettsii* and the 115- and 135-kDa polypeptides of *R. conorii*, as demonstrated by Western immunoblotting technique. Further elucidation of epitope mapping of these important surface protein antigens will require other experimental approaches.

MAbs against the species-specific antigens may be used to identify the species of SFG rickettsiae. They might also be employed in the preparation of species-specific antigens by affinity chromatography. Isolation of these epitopes or preparation of anti-idiotype antibodies to the species-specific

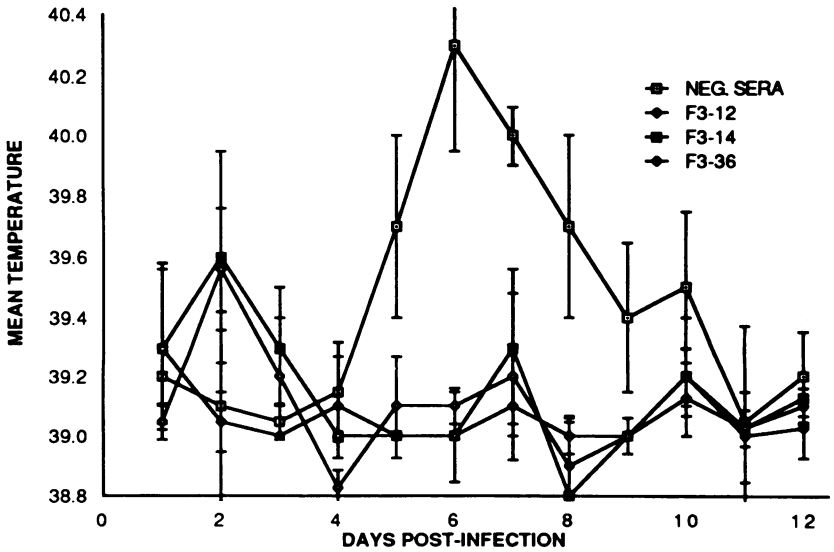

FIG. 6. Mean rectal temperatures (°C) of guinea pigs inoculated with mixtures of 10 median febrile doses of *R. rickettsii* and MAbs F3-12, F3-14, F3-36, or normal mouse (NEG.) serum as a control. Each point represents the mean temperature of three guinea pigs.

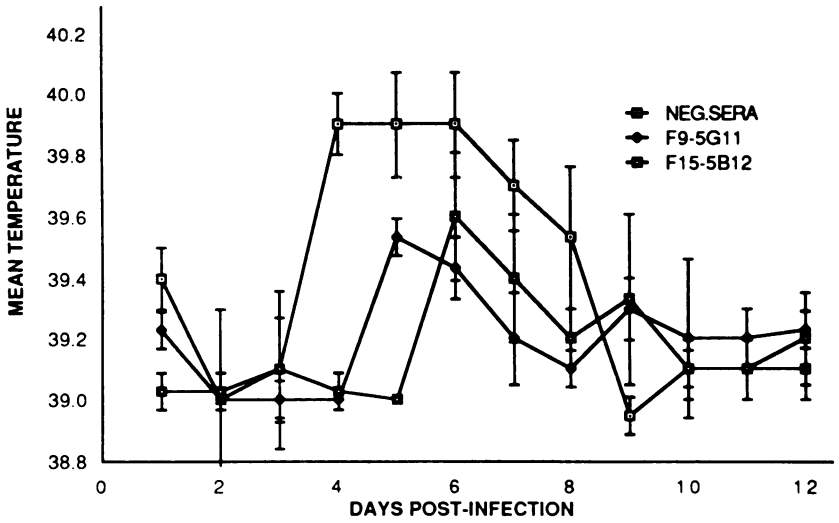

FIG. 7. Mean rectal temperatures of guinea pigs inoculated with mixtures of 10 median febrile doses of *R. sibirica* and MAbs F9-5G11, F15-5B12, or normal mouse (NEG.) serum as a control. Each point represents the mean temperature of three guinea pigs.

MAbs would allow development of species-specific serologic assays for diagnosis and epidemiologic studies. Furthermore, these MAbs confer on animals protection against febrile diseases caused by *R. rickettsii*, *R. sibirica*, and *R. conorii* (11, 16). Thus, the heat-sensitive polypeptides with which the MAbs against species-specific antigens react are important candidate vaccinogens. The MAbs will also be useful in analyzing recombinant clones of rickettsial DNA for expression of important antigens.

The mechanisms by which MAbs protect animals from infection may include opsonization and consequent enhancement of rickettsial phagocytosis by macrophages. Since MAbs against some surface constituents such as LPS-like antigens do not protect animals from infection (1, 2, 4, 11), opsonization may not be the only protective mechanism. Another hypothesis is that the MAb may block either rickettsial attachment to the host cell or the entry mechanism, for example, by binding to a rickettsial protein that mediates attachment or by inhibiting the activity of one or more enzymes which are required for entry into the host cells. McDonald et al. (17) recently reported the construction of a genomic library of *R. rickettsii* recombinant DNA.

TABLE 3. Results of challenge of susceptible mice with virulent *R. conorii* that had been reacted with selected MAbs

Inoculum	No. of mice[a] showing:		% Morbidity	% Mortality
	Illness[b]	Death		
MAbs				
F2-15	0	0	0	0
F2-31	0	0	0	0
F2-53	0	0	0	0
F3-12	0	0	0	0
F3-47	4	3	80	60
Control serum[c]	5	4	100	80

[a] Each group tested contained five mice.
[b] Illness indicated by inactivity, ruffled fur, and loss of body weight.
[c] Mortality and morbidity of control group versus groups F2-15, F2-31, F2-53, and F3-12 ($P < 0.01$); group F3-47 did not differ from the control group ($P > 0.05$) by the F test.

A heat-sensitive rickettsial protein expressed by one of the clones reacted with MAbs that neutralized the mouse toxicity phenomenon, and this recombinant DNA-expressed antigen stimulated animals to resist challenge with *R. rickettsii* (17). The molecular size of this antigen appeared to be 130 kDa in the native state and 155 kDa after heat modification of electrophoretic mobility. Similar proteins were noted in our study and may be useful candidates for similar studies. The development of protective species-specific and group-reactive MAbs will assist in the evaluation of the crucial antigenic determinants of immunity and the development of effective vaccines against pathogenic SFG rickettsiae. It is of equal importance that the MAbs be used to elucidate rickettsial physiologic and pathologic mechanisms.

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LITERATURE CITED

1. **Anacker, R. L., R. H. List, R. E. Mann, S. F. Hayes, and L. A. Thomas.** 1985. Characterization of monoclonal antibodies protecting mice against *Rickettsia rickettsii*. J. Infect. Dis. **151:** 1052–1060.
2. **Anacker, R. L., R. H. List, R. E. Mann, and D. L. Wiedbraunk.** 1986. Antigenic heterogeneity in high- and low-virulence strains of *Rickettsia rickettsii* revealed by monoclonal antibodies. Infect. Immun. **51:**653–660.
3. **Anacker, R. L., R. E. Mann, and C. Gonzales.** 1987. Reactivity of monoclonal antibodies to *Rickettsia rickettsii* with spotted fever and typhus group rickettsiae. J. Clin. Microbiol. **25:**167–171.
4. **Anacker, R. L., G. A. McDonald, R. H. List, and R. E. Mann.** 1987. Neutralizing activity of monoclonal antibodies to heat-sensitive and heat-resistant epitopes of *Rickettsia rickettsii* surface proteins. Infect. Immun. **55:**825–827.
5. **Anacker, R. L., R. N. Philip, E. Casper, W. J. Todd, R. E. Mann, M. R. Johnston, and C. J. Nauck.** 1983. Biological properties of rabbit antibodies to surface antigen of *Rickettsia rickettsii*. Infect. Immun. **40:**292–298.
6. **Craigie, J. D., W. Watson, E. M. Clark, and M. E. Malcomson.**

1945. The serological relationships of the rickettsiae of epidemic and murine typhus. Can. J. Res. Sect. E Med. Sci. **24**:84–103.

7. **Dasch, G. A.** 1981. Isolation of species-specific protein antigens of *Rickettsia typhi* and *Rickettsia prowazekii* for immunodiagnosis and immunoprophylaxis. J. Clin. Microbiol. **14**:333–341.
8. **Dasch, G. A., J. P. Burans, M. E. Dobson, F. M. Rollwagen, and J. Misiti.** 1984. Approaches to subunit vaccines against the typhus rickettsiae, *Rickettsia typhi* and *Rickettsia prowazekii*, p. 251–256. *In* L. Leive and D. Schlessinger (ed.), Microbiology–1984. American Society for Microbiology, Washington, D.C.
9. **Dasch, G. A., J. R. Samms, and J. C. Williams.** 1981. Partial purification and characterization of the major species-specific protein antigens of *Rickettsia typhi* and *Rickettsia prowazekii* identified by rocket immunoelectrophoresis. Infect. Immun. **31**: 276–288.
10. **Fan, M.-Y., J.-G. Wang, Y.-X. Jiang, D.-G. Zong, B. Lenz, and D. H. Walker.** 1987. Isolation of a spotted fever group rickettsia from a patient and related ecologic investigations in Xinjiang Uygur Autonomous Region of China. J. Clin. Microbiol. **25**: 628–632.
11. **Feng, H. M., D. H. Walker, and J. G. Wang.** 1987. Analysis of T-cell-dependent and -independent antigens of *Rickettsia conorii* with monoclonal antibodies. Infect. Immun. **55**:7–15.
12. **Fulton, F., and A. M. Begg.** 1946. Chemotherapeutic and other studies of typhus. V. The antigenic structure of typhus rickettsiae. Med. Res. Counc. (G.B.) Spec. Rep. Ser. **255**:163–191.
13. **Gelsema, W. J., and C. L. Deligny.** 1977. Isoelectric focusing as a method for the characterization of ampholytes. J. Chromatogr. **130**:41–55.
14. **Hanson, B. A., C. L. Wisseman, Jr., A. Waddell, and D. J. Silverman.** 1981. Some characteristics of heavy and light bands of *Rickettsia prowazekii* on Renografin gradients. Infect. Immun. **34**:596–604.
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
16. **Lange, J. V., and D. H. Walker.** 1984. Production and characterization of monoclonal antibodies to *Rickettsia rickettsii*. Infect. Immun. **46**:289–294.
17. **McDonald, G. A., R. L. Anacker, and K. Garjlan.** 1987. Cloned gene of *Rickettsia rickettsii* surface antigen: candidate vaccine for Rocky Mountain spotted fever. Science **235**:83–85.
18. **Philip, R. N., E. A. Casper, W. Burgdorfer, R. K. Gerloff, L. E. Hughes, and E. J. Bell.** 1978. Serologic typing of rickettsiae of the spotted fever group by microimmunofluorescence. J. Immunol. **121**:1961–1968.
19. **Shepard, C. C.** 1945. Typhus fever: antigen of the rickettsiae of typhus fever and the changes produced by heat. N.I.H. Bull. **183**:93–110.
20. **Wang, J.-G., D. H. Walker, H. Li, B. Lenz, and T. R. Jerrells.** 1987. Barbash strain spotted fever group rickettsia is a strain of *Rickettsia conorii* and differs from *Rickettsia sibirica*. Acta Virol. (Prague) (Engl. Ed.) **31**:489–498.