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Addition of Monoclonal Antibodies Specific for *Rickettsia akari* to the Rickettsial Diagnostic Panel

JOSEPH E. McDADE,[1]* CHARLOTTE M. BLACK,[2] L. FLORETTA ROUMILLAT,[1] MARTHA A. REDUS,[1] AND CATHERINE L. SPRUILL[1]

Division of Viral Diseases[1] and Division of Host Factors,[2] Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

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Monoclonal antibodies were produced from mice infected with *Rickettsia akari* (the etiologic agent of rickettsialpox) and evaluated for specificity in indirect fluorescent-antibody tests with 23 different rickettsial antigens. Of the nine antibodies that were evaluated, two were specific for *R. akari* and four reacted with *R. akari* and all other spotted fever group rickettsiae. The remaining three antibodies reacted with some, but not all, members of the spotted fever group. None of the antibodies reacted with typhus, scrub typhus, trench fever, or Q fever rickettsiae. Adding these antibodies to the list of available diagnostic reagents will facilitate identification of rickettsial diseases, particularly those caused by members of the spotted fever group, where the clinical presentations are similar and the etiologic agents are closely related antigenically.

The rickettsiae are divided into five serogroups—spotted fever, typhus, scrub typhus, Q fever, and trench fever—on the basis of the presence of common antigens among member species (4). Convalescent-phase sera from patients infected with a given species react with other species in the same serogroup, but, with certain exceptions (11), they rarely cross-react with species in the other serogroups.

The antigenic relatedness of rickettsiae can confound attempts to determine the specific etiology of rickettsial infections. For example, the spotted fever group is comprised of five known pathogens—*Rickettsia rickettsii* (Rocky Mountain spotted fever), *Rickettsia conorii* (boutonneuse fever), *Rickettsia sibirica* (Siberian tick typhus), *Rickettsia australis* (Australian tick typhus), and *Rickettsia akari* (rickettsialpox)—and the clinical manifestations of the various illnesses are frequently indistinguishable. Complement fixation tests with species-specific antigens or toxin neutralization tests can identify the specific etiologic agent (4), but reagents for these tests are expensive to produce and standardize and are not generally available. Thus, in recent years, the etiology of most spotted fever group infections has been determined by a combination of clinical and epidemiologic parameters, together with the results of group-specific serologic tests; patients whose sera have diagnostic titers to spotted fever group antigens and who have a clinically compatible illness are presumed to have been infected with the agent enzootic in a given area.

Accumulating evidence, however, suggests that the geographic ranges of various spotted fever group rickettsiae overlap to a far greater extent than previously appreciated, and that the practice of identifying etiologic agents by epidemiologic criteria is not always valid. For example, Rocky Mountain spotted fever is presumed to be confined to the Western Hemisphere, but reports from Israel (5, 6) suggest that a similar disease exists there as well. Although the prevailing opinion is that Israeli spotted fever is caused by unusual strains of *R. conorii*, the precise etiology remains obscure. Similarly, Yamamoto et al. (13) recently reported that spotted fever infections of undetermined etiology occurred in Japan.

* Corresponding author.

An appropriate panel of monoclonal antibodies would facilitate the laboratory diagnosis of rickettsial infections. Not only would such antibodies allow the direct identification of isolates from patients, but they could also be used in inhibition tests with sera so that the respective infections could be diagnosed serologically. Additionally, monoclonal antibodies are useful probes for researchers who are attempting to produce specific rickettsial antigens by recombinant DNA technology. Monoclonal antibodies specific for *R. rickettsii* (1, 7), *Rickettsia tsutsugamushi* (scrub typhus) (3, 8), *Coxiella burnetii* (Q fever) (12), and *Rickettsia prowazekii* (epidemic typhus) (2) have already been reported, but to date no reports have appeared of monoclonal antibodies specific for *R. akari*, *R. australis*, *R. sibirica*, or *Rickettsia typhi* (murine typhus). We have produced monoclonal antibodies specific for *R. akari* to help complete the rickettsial diagnostic panel; they are available in limited quantity and can be obtained by contacting the principal author.

Production of monoclonal antibodies. BALB/c mice were infected with dilute yolk sac suspensions of the MK strain of *R. akari*. The spleens were harvested from these animals 6 days later, homogenized into 10% suspensions in phosphate-buffered saline (pH 7.2), and passaged into a second group of mice. The latter mice were given oral tetracycline when they became ill 4 days following infection. The surviving mice in the latter group received booster immunizations 2 months later (3 days prior to hybridoma production); each animal was inoculated intravenously with 0.5 ml of inactivated *R. akari* cells (optical density at 660 nm, 0.05) that had been purified by an established sequential centrifugation procedure (2). Hybridomas were produced by fusing immune spleen cells with mouse myeloma cell line SP2/0 as described previously (2). The methods that we used to select and clone antibody-producing hybridomas and to purify antibodies have also been described previously (2).

Evaluation of the specificities of the monoclonal antibodies. The rickettsiae that were tested, the original sources of the respective strains, and their method of propagation are shown in Table 1. Rickettsiae were inactivated with gamma irradiation (total dose, 200,000 rads) and homogenized in phosphate-buffered saline to form 10% suspensions; these suspensions served as stock antigen preparations. The opti-

TABLE 1. Strains of rickettsiae tested with monoclonal antibodies

Species	Strain	Original source[a]	Passage history[b]	Method of propagation
Rickettsia rickettsii	Sheila Smith	Human	?/multiple passage in CE	Yolk sac
	Costa Rican	Human	1 GP/3 CE/2 GP/2 CE	Yolk sac
	JW	Human	1 GP/2 CE/2 Vero	Vero cells
	Hlp	*Haemaphysalis leporis palustris*	51 CE/9 TC/4 CE/3 Vero	Vero cells
R. conorii	Moroccan	Unknown	Numerous GP/292 CE/5 Vero/2 CE	Yolk sac
	Indian tick typhus	Ticks (species unknown)	50+ GP/11 CE/4 Vero	Vero cells
R. montana	ATCC VR-611	*Dermacentor* sp.	22 CE	Yolk sac
R. bellii	369-C	*Dermacentor variabilis*	1 T/2 CE/2 TC/1 CE/13 TC	Vero cells
R. rhipicephali	3-7-06	*Rhipicephalus sanguineus*	?/4 Vero CDC lab	Vero cells
R. slovaca	D	*Dermacentor marginatum*	26 CE/3 Vero	Vero cells
R. sibirica	246	*Dermacentor nuttalli*	?/20 CE	Yolk sac
Thai tick typhus	TT-118	Pool of *Ixodes* and *Rhipicephalus* ticks	1 GP/8 CE/4 Vero	Vero cells
R. australis	Phillips	Human	122 CE/2 Vero	Vero cells
R. akari	MK	Human	2 M/12 CE/1 GP/8 CE	Yolk sac
	Hartford		?/multiple CE CDC lab	Yolk sac
R. prowazekii	Breinl	Human	?/multiple CE CDC lab	Yolk sac
R. typhi	Wilmington	Human	?/multiple CE CDC lab	Yolk sac
R. canada	McKiel	*Haemaphysalis leporis palustris*	?/multiple CE CDC lab	Yolk sac
R. tsutsugamushi	Karp	Human	?/multiple CE CDC lab	Yolk sac
	Gilliam	Human	?/multiple CE CDC lab	Yolk sac
R. quintana	Fuller	Human	?/multiple passages in supplemented blood agar	Blood agar
Coxiella burnetti	Nine Mile	*Dermacentor andersoni*	Phase II antigen-multiple GP multiple CE passage	
	California 76	Cow's milk	Phase I antigen—3 CE	Yolk sac

[a] Specific names indicate various species of ticks from which isolates were made. For additional information, see reference 11.
[b] Abbreviations: CDC, Centers for Disease Control; CE, chick embryo; GP, guinea pig; M, mouse; T, tick; TC, tissue culture (cell type unspecified); ?, history incomplete.

TABLE 2. Reactivity of *R. akari* monoclonal antibody panel with selected rickettsiae

Serogroup and organism (strain)	Reactivity[a] with monoclonal antibody								
	78-26-4	77-244-20	78-108-19	78-26-14	78-22-5	78-142-11	78-7-19	78-142-17	78-142-3
Spotted fever									
Rickettsia akari (Hartford)	+	+	+	+	+	+	+	+	+
R. akari (MK)	+	+	+	+	+	+	+	+	+
R. rickettsii (Costa Rican)	−	−	−	−	−	+	+	+	+
R. rickettsii (Sheila Smith)	−	−	−	−	−	+	+	+	+
R. rickettsii (Hlp)	−	−	−	−	−	+	+	+	+
R. rickettsii (JW)	−	−	−	−	−	+	+	+	+
R. conorii (Moroccan)	−	−	−	−	−	+	+	+	+
R. conorii (Indian tick typhus)	−	−	−	−	−	+	+	+	+
Thai tick typhus	−	−	−	±	+	+	+	+	+
R. sibirica	−	−	±	±	±	+	+	+	+
R. australis	−	−	−	±	−	+	+	+	+
R. montana	−	−	−	−	−	±	+	+	+
R. rhipicephali	−	−	−	±	+	+	+	+	+
R. slovaca	−	−	−	±	±	+	+	+	+
R. bellii	−	−	−	−	−	−	−	−	−
Typhus									
R. prowazekii	−	−	−	−	−	−	−	−	−
R. typhi	−	−	−	−	−	−	−	−	−
R. canada	−	−	−	−	−	−	−	−	−
Scrub typhus									
R. tsutsugamushi (Karp)	−	−	−	−	−	−	−	−	−
R. tsutsugamushi (Gilliam)	−	−	−	−	−	−	−	−	−
Trench fever, *R. quintana*	−	−	−	−	−	−	−	−	−
Q fever									
Coxiella burnetii (Nine Mile) phase I	−	−	−	−	−	−	−	−	−
C. burnetii (Nine Mile) phase II	−	−	−	−	−	−	−	−	−

[a] +, Reactivity; −, no reactivity; ±, minimal, with few organisms.

mum working dilutions of each suspension were then determined by direct or indirect fluorescent-antibody tests with appropriate (polyvalent) immune sera.

In our initial determinations of antibody reactivity, the monoclonal antibodies were titrated with *R. akari* antigen by the indirect fluorescent-antibody technique (11). Because all antibodies reacted with *R. akari* at titers ranging from 64 to >512, subsequent tests with other antigens were performed at a single (1:10) dilution.

Two monoclonal antibodies (78-26-4 and 77-244-20) apparently were specific for *R. akari* (Table 2). Only two strains of *R. akari* were tested, and so it is possible that tests with the few additional isolates that are available would fail to confirm their specificity. However, *R. akari* is relatively distinct antigenically in tests with polyvalent mouse antisera (11), and therefore it is likely that additional testing will confirm the specificity of the monoclonal antibodies. The use of plaque-purified rickettsiae as immunogens and test antigens would contribute significantly to such specificity determinations.

Four other antibodies (78-142-11, 78-7-19, 78-142-17, and 78-142-2) reacted with all members of the spotted fever group. The three remaining antibodies reacted with some, but not all, spotted fever group rickettsiae (Table 2). None of the antibodies reacted with species in the typhus, scrub typhus, trench fever, or Q fever groups. Of interest was the fact that the method of cultivating rickettsiae did not affect their reactivity. Different strains of the same species reacted similarly with the respective antibodies. Of particular interest was the uniform lack of reactivity of any antibodies with *Rickettsia bellii*. This species had been provisionally placed in the spotted fever group (10), but it also has properties in common with typhus group rickettsiae (9). Our findings suggest that antigenically *R. bellii* is more distinct from the spotted fever group than perhaps has heretofore been appreciated.

We encourage others who may have additional relevant monoclonal antibodies in their repertoire of reagents to notify the scientific community so that the rickettsial diagnostic panel can be completed.

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