Cross-reaction of immune sera from patients with rickettsial diseases

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Summary. *Rickettsia rickettsii* and *Rickettsia conorii* are the causative agents of two common and serious diseases, Rocky Mountain spotted fever and Mediterranean spotted fever, respectively. In patients naturally infected with either of these organisms, antibodies are produced which cross-react with antigens of the other so extensively that diagnostic tests usually cannot identify the causative agents. The results of this study indicate that serodiagnostic tests with antigen from one of these two organisms could be used to detect antibodies in patients with either of the two rickettsial diseases.

Introduction

Cross-reactions among rickettsiae of the spotted fever group have been known for many years. *Rickettsia conorii*, the causative agent of Mediterranean spotted fever, when injected into guinea-pigs, protects the animals against challenge with *Rickettsia rickettsii* (Badger, 1933; Walker *et al.*, 1984). Plotz *et al.* (1944) subsequently showed that convalescent guinea-pig sera cross-reacted with antigen obtained from patients with Mediterranean spotted fever and Rocky Mountain spotted fever. The homologous titres with either antigen were significantly higher than the heterologous titres. Feng and Waner (1980) demonstrated that guinea-pigs immunised with *R. montana* produced antibodies that cross-reacted with *R. rickettsii* antigens and that protected the animal from fatal infection with *R. rickettsii*. In contrast, Pickens *et al.* (1965) and Lackman *et al.* (1965) demonstrated species specific-antibodies in mice. Philip *et al.* (1978) also showed little cross-reaction in microimmunofluorescence (micro-IF) tests when antisera from mice inoculated with *R. rickettsii* were tested against *R. conorii*, or when antisera from mice inoculated with *R. conorii* were tested against *R. rickettsii*.

Although the cross-reactivity of antibodies to rickettsiae of the spotted fever group in human sera is extensive, few reports have been published. Plotz *et al.* (1944) showed cross-reaction with *R. conorii* and *R. rickettsii* antigens in complement-fixation

tests of sera from 15 patients, ten of whom had Rocky Mountain spotted fever and five Mediterranean spotted fever. Philip *et al.* (1976) showed by immunofluorescence that sera from seven patients with Rocky Mountain spotted fever cross-reacted with *R. conorii*. The heterologous titres in both the complement fixation and immunofluorescence tests were significantly lower than the homologous titres.

In an evaluation of the specificity of a newly developed latex test for the detection of antibodies to *R. conorii* in patients with Mediterranean spotted fever (Hechemy *et al.*, 1986), we also observed extensive cross-reaction with *R. rickettsii* expressed qualitatively as percentage of reactive sera. The heterologous antibody titres were mostly similar to those obtained in the homologous reaction.

We now describe our observations on the cross-reactions of human sera from patients with Mediterranean spotted fever, Rocky Mountain spotted fever, or murine typhus, with the heterologous rickettsial antigens by use of three common serodiagnostic tests.

Materials and methods

Antigens

R. conorii (Moroccan strain) and *R. rickettsii* (Sheila Smith) were grown in Vero cells, purified as described by Weiss *et al.* (1975) and lyophilised. *R. typhi* (Wilmington) was a gift from Dr T. Tzianabos, Centers for Disease Control, Atlanta, GA, USA. Erythrocyte-sensitising

substance from each organism was prepared as described by Anacker *et al.* (1975).

Sera

Sera of patients with Mediterranean spotted fever (56) were selected from positive samples submitted to the Marseille laboratory. Sera of patients with Rocky Mountain spotted fever (11) or murine typhus (12) were obtained from samples submitted to the Texas laboratory.

Serological tests

The serological reagents were prepared, and the tests performed, as described by Philip *et al.* (1976) (Micro-IF), Anacker *et al.* (1975) (indirect haemagglutination test; IHA), and Hechemy *et al.* (1986) (latex test). The optimum concentrations of the erythrocyte-sensitising substances from the three antigen preparations used in the IHA and latex tests were determined by chessboard titration. This was done with a human serum pool reactive to each of the three rickettsial antigens in micro-IF tests. The micro-IF/Ig refers to the micro-IF test in which fluorescein isothiocyanate conjugate-specific goat antibody to human IgA + IgG + IgM (heavy + light chains) was used; micro-IF/IgG refers to goat antibody to human IgG (γ chain); micro-IF/IgM indicates goat antibody to human IgM (μ chain). The minimum significant levels of reactivity (Philip *et al.*, 1976; Hechemy *et al.*, 1986) for each test were previously determined. The minimum significant levels of reactivity for the micro-IF tests with the fluorescein isothiocyanate conjugate specific for IgA + IgG + IgM, IgG and IgM were titres of 128, 128 and 32 respectively. For the indirect haemagglutination and latex tests, the minimum titres were 128 and 64 respectively.

Statistical analysis

Fisher's exact test was used to compare sera reactive with presumed "homologous" and presumed "heterologous" antigens.

Results

The qualitative test results (table I) indicated extensive cross-reaction among antigens of rickettsiae of the spotted fever group by all three tests. Cross-reaction of sera was less extensive with murine typhus group antigen than with the other antigens, especially in sera from patients with Rocky Mountain spotted fever.

Sera from patients with murine typhus cross-reacted more with *R. conorii* than with *R. rickettsii* antigens, although the sample size was too small to assess statistical significance. Those sera also appeared to exhibit cross-reactions relatively more in micro-IF/IgM, latex, and IHA tests than in the micro-IF/IgG test.

Titration of sera from patients with Mediterranean spotted fever with rickettsial antigens showed that the titres (table II) were within one twofold dilution for 88% of the 56 sera tested by micro-IF/Ig and micro-IF/IgG and for 60% tested by micro-IF/IgM, IHA and latex. For 12 of the sera higher titres were obtained with the heterologous *R. rickettsii* antigens by the IHA tests. With the 11 sera from patients with Rocky Mountain spotted fever, the titres with the heterologous *R. conorii* antigen were within one two-fold dilution for 10, 10, eight and 11 sera in the micro-IF/Ig, micro-IF/

Table I. Qualitative cross-reactivity of reactive sera with heterologous antigens in three tests

Diagnosis	Heterologous antigen	Homologous antigen	Number of sera reactive with heterologous/homologous antigen in				
			Micro-IF				
			Ig	IgG	IgM	Latex test	IHA
Mediterranean spotted fever (n = 56)	<i>R. rickettsii</i>	<i>R. conorii</i>	52/56	44/47	34/49	48/56	55/56
	<i>R. typhi</i>	<i>R. conorii</i>	6/56	3/47	4/49	11/56	7/56
Rocky Mountain spotted fever (n = 11)	<i>R. conorii</i>	<i>R. rickettsii</i>	8/11	7/7	6/7]*	11/11	11/11
	<i>R. typhi</i>	<i>R. rickettsii</i>	0/8†	0/7	3/6†	2/11	1/11
Murine typhus (n = 13)	<i>R. conorii</i>	<i>R. typhi</i>	2/12]*	1/7]*	5/13]*	8/13	7/13]*
	<i>R. rickettsii</i>	<i>R. typhi</i>	0/12]	0/7]	2/13]	2/13	8/13]

Micro-IF = microimmunofluorescence test.

IHA = indirect haemagglutination test.

* Difference was not statistically significant ($p > 0.05$) (Fisher's exact test).

† Insufficient serum to complete all the micro-IF tests on all specimens.

Table II. Quantitative cross-reactivity of 56 sera from patients with Mediterranean spotted fever sera with the heterologous *R. rickettsii* antigen

Test	Number of sera in which the heterologous: homologous titre ratio was		
	≤ 0.25	0.5-2	≥ 4
Micro-IF			
Ig	5	49	2
IgG	6	50	0
IgM	24	32	0
IHA	9	35	12
Latex	21	33	2

Micro-IF = microimmunofluorescence test; IHA = indirect haemagglutination test.

IgG, IgM and IHA tests respectively. In contrast, titres of only four sera were within one two-fold dilution in the latex test. For all sera in which cross-reactions between spotted fever group and typhus group antigens were observed, the titres exhibited at least a four-fold difference. However, the sera from patients with murine typhus displayed relatively higher titres to *R. conorii* than to *R. rickettsii*.

Discussion

Rickettsial diseases, especially murine typhus, Mediterranean spotted fever and Rocky Mountain spotted fever continue to constitute an important public health problem in certain areas of the world. The production of tests for the serodiagnosis of each of the separate rickettsial diseases is not cost effective. This is because the number of patients is relatively small, the cost of preparation of the diagnostic reagents is high, and the antibiotic therapy is the same regardless of the specific rickettsial aetiology. Therefore, we investigated the cross-reactivity of sera from patients diagnosed as having rickettsial spotted fever or murine typhus in

three tests which are currently the mainstay for the serodiagnosis of these diseases.

In the micro-IF test the whole organism is used to detect a range of rickettsial antigens. In contrast the latex and IHA tests make use of an NaOH-solubilised extract of rickettsial organism composed mainly of carbohydrate or carbohydrate-containing fractions, and detect antibodies only to these fractions.

Cross-reactivity between the spotted fever group and the murine typhus group was not extensive and even when cross-reaction occurred, the differences in titre were large enough to differentiate between infection with the two rickettsial groups.

In contrast, extensive cross-reactions were observed within the spotted fever group, both qualitatively (number of sera) and quantitatively (titres). Considered quantitatively, the micro-IF/Ig and micro-IF/IgG tests, were relatively "less specific" than the other tests for the differentiation of Rocky Mountain spotted fever from Mediterranean spotted fever. This was not unexpected. The whole array of proteins and polysaccharide antigens on the organism is available to react with the antisera in the micro-IF test, whereas the protein components are excluded from the latex and IHA tests because of the NaOH treatment.

Because of the similarity of homologous and heterologous titres, infection with *R. rickettsii* and *R. conorii* organism cannot usually be differentiated by the standard diagnostic technique (Elisberg and Bozeman, 1979) unless the geographical origin of the infection is known. Thus 12 cases of Mediterranean spotted fever would have been diagnosed as Rocky Mountain spotted fever if sera had been submitted in the USA and tested by the IHA test. Conversely, the micro-IF/Ig, micro-IF/IgG or the IHA tests with either *R. rickettsii* or *R. conorii* antigens could be used for the serodiagnosis of either disease.

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