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Short Communication

Serological cross-reactivity between spotted fever and typhus groups of rickettsia infection in Japan

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ABSTRACT

Objectives: We examined the frequency of cross-reactions to *Rickettsia typhi* in patients with Japanese spotted fever (JSF) and evaluated the differences between two rickettsiae using antibody endpoint titers.
Methods: Patients' immunoglobulin (Ig)M and IgG titers against *Rickettsia japonica* and *Rickettsia typhi* in two phases were measured using an indirect immunoperoxidase assay at two reference centers for rickettsiosis in Japan. Cross-reaction was defined as a higher titer against *R. typhi* in convalescent sera than in acute sera among patients fulfilling the criteria for JSF diagnosis. The frequencies of IgM and IgG were also evaluated.
Results: Approximately 20% of cases showed positive cross-reactions. A comparison of antibody titers revealed the difficulty in identifying some positive cases.
Conclusion: Cross-reactions of 20% in serodiagnosis may lead to the misclassification of rickettsial diseases. However, with the exception of some cases, we were able to successfully differentiate JSF from murine typhus using each endpoint titer.

Introduction

Rickettsioses are caused by the transmission of intracellular Gram-negative bacteria of the genus *Rickettsia* from arthropods to humans. They are classified into two groups, namely the spotted fever group (SFG) and the typhus group (TG) [1]. Although SFG and TG rickettsiae are predominantly transmitted by ticks and fleas, respectively, both infections have similar manifestations. Thus, their diagnosis relies mainly on serological tests [1].

Conventional serodiagnosis using indirect immunofluorescence assay (IFA) or indirect immunoperoxidase (IP) assay in paired sera remains a cornerstone for acute rickettsial infections [2]. However, serological cross-reactions between different rickettsia groups can occur because of the production of antibodies that recognize proteins with the same antigenicity [3]. These cross-reactions can lead to misdiagnosis, especially in patients with non-specific clinical manifestations, unless physicians differentiate the rickettsia groups by comparing the respective rickettsial antibody titers in the acute and convalescent sera and by physical findings, such as eschars [2].

Although cross-reactivity within SFG has been investigated [4], cross-reactivity between SFG and TG has not been fully evaluated. The organisms responsible for cross-reactivity between SFG and TG in Japan today are *Rickettsia japonica*, known as Japanese spotted fever (JSF), and *Rickettsia typhi*, known as murine typhus (MT). Uchiyama et al. [3] identified cross-reactive antibodies of *R. typhi* against *R. japonica* in patients with JSF using western blotting and cross-absorption. Nonetheless, these assays are difficult to implement as standard diagnostic tests because they require expertise. Clinicians must consider cross-reactions based on clinical features and conventional serological results in paired sera. Although experts have proposed that the variation of each rickettsial antibody titer in the two phases differentiates a true infection from a

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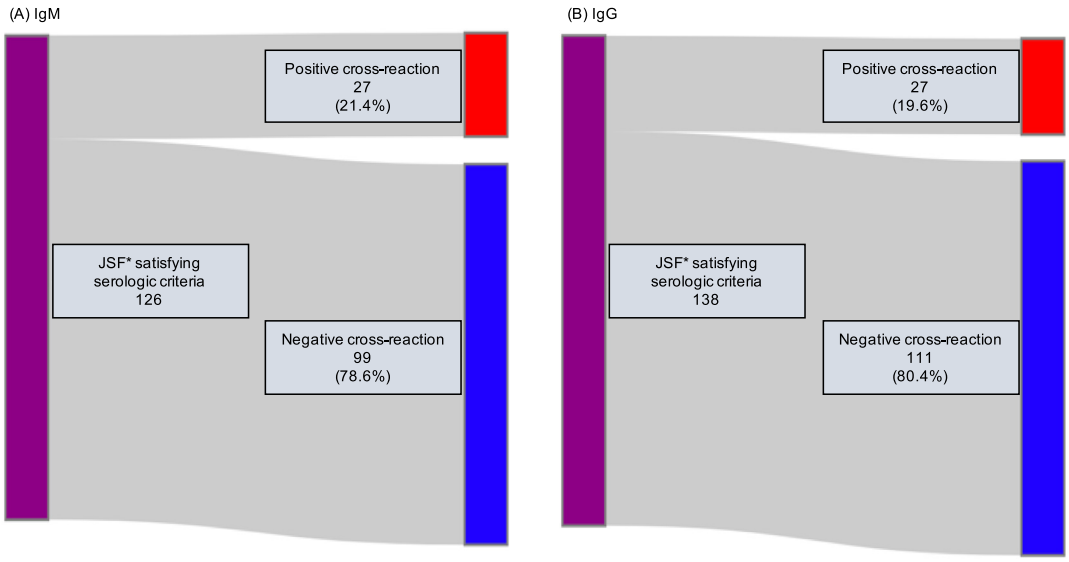

Figure 1. Cross-reactions against *Rickettsia typhi* in patients with JSF: (a) IgM and (b) IgG. Of the 145 cases analyzed, 119 underwent a ≥4-fold increase in titers against *Rickettsia japonica* or the occurrence of seroconversion in paired sera for both IgM and IgG, with seven and 19 showing these serological reactions only for IgM and IgG, respectively (total number of analyzed cases with IgM, 126; those with IgG, 138).
Ig, immunoglobulin; JSF, Japanese spotted fever.

cross-reaction [5], no clinical evidence is available to support this. Using a conventional method, we investigated the cross-reactions between *R. japonica* and *R. typhi* in paired sera from patients with a clinically and serologically confirmed *R. japonica* infection.

Methods

We retrospectively examined serological data from two reference centers for rickettsiosis in Japan between January 01, 2003, and December 31, 2016. All patient samples were tested by IP using the Aoki strain of *R. japonica* and the Wilmington strain of *R. typhi* (Appendix). IP has a specificity comparable to that of IFA and represents an alternative standard serological diagnostic test for rickettsioses [6]. Patients with JSF were defined as having clinical features of the disease and a ≥4-fold increase in immunoglobulin (Ig)M or IgG titers against *R. japonica* or seroconversion (defined as titers ≥80) in paired sera [7].

Cross-reaction was defined as a higher titer against *R. typhi* in convalescent sera than in acute sera among patients fulfilling the criteria for JSF diagnosis. The frequencies were calculated for IgM and IgG. This definition was developed to overcome the difficulty in differentiating between previous *R. typhi* infections and cross-reactions using only monophasic sera [4]. We also examined cross-reactivity among patients with JSF, which was confirmed by positive combination tests of conventional polymerase chain reaction (PCR) and the sequencing analysis of *R. japonica* using blood and skin specimens and/or positive immunohistochemistry for SFG rickettsia using skin biopsy specimens, such as eschars and erythematous lesions [8]. These positive results were reported by the physicians in charge of the cases. Additionally, patient samples that showed cross-reactivity but posed challenges in being identified as either JSF or MT based on IP alone were subjected to indirect hemagglutination tests (HA) [9].

Results

In total, 145 patients with JSF were analyzed. The median interval between acute and convalescent serum collection was 13 days (interquartile range: 8-17 days). Changes in titers for *R. japonica* and *R. typhi* are shown in Appendix Figure A1. Cross-reactions against *R. typhi* in patients with JSF were observed in 21.4% of the cases (27/126, 95% confidence interval [CI] 14.6-29.6) for IgM and 19.6% (27/138, 95% CI 13.3-27.2) for IgG (Figure 1). IgM and IgG titers are shown in Table 1. In 11 cases of JSF confirmed by PCR and/or immunohistological tests, cross-reactions were observed in 18.2% of cases (2/11, 95% CI 2.3-51.8) each for IgM and IgG (Appendix, Table A1). Eight cases could not be identified as true infections or cross-reactions by comparing the endpoint titer levels of the two rickettsiae; of these, seven cases could be differentiated using HA (Appendix, Tables A2 and A3).

Discussion

Using conventional serological methods with paired sera, we found that the proportion of cross-reactions between *R. japonica* and *R. typhi* in patients with JSF was approximately 20%, suggesting that some degree of misclassification in serodiagnosis does occur. The robustness of our results was supported by a similar frequency of cross-reactions in patients with JSF, confirmed using PCR and/or immunohistological skin findings.

A previous study identified cross-reactions between *R. japonica* and *R. typhi* in patients with JSF using IFA, immunoblotting, and cross-absorption assays, and focused on identifying proteins with common antigenicity using a small sample size [3]. Our study is the first to estimate the frequency of cross-reactions using a large number of patients with JSF and evaluate distinguishability using changes in antibody titers.

The value of endpoint antibody titers has been proposed to differentiate SFG from TG [4,5], and this proposition has been partially supported by our research. In fact, using a case definition of clinically and serologically confirmed patients with JSF, we were able to distinguish between these rickettsial infections in most patients by comparing each endpoint titer in the acute and convalescent sera. However, the presence of cross-reactions showing equal or higher endpoint titers in *R. typhi* compared to *R. japonica* highlights the need for further studies on the diagnostic ability of endpoint titers among rickettsiae.

Table 1
Antibody titers against *Rickettsia japonica* and *Rickettsia typhi* in paired sera showing cross-reactivity. (a) IgM titers of sera showing cross-reactivity with IgM and (b) IgG titers of sera showing cross-reactivity with IgG. The titer was expressed as the reciprocal of the highest dilution of serum, which demonstrated blue or blue-black-colored rickettsial particles (the reciprocal of titers of 0 indicates <1:40).

(a)

ID	Interval (days)	*R. japonica*		*R. typhi*	
		Acute	Convalescent	Acute	Convalescent
1005	8	80	320	0	40
1007	14	0	1280	80	640
1017	16	0	160	0	40
1019	18	0	640	0	40
1020	10	0	320	0	160
1027	22	0	320	0	160
1033	15	0	320	0	40
1036	10	0	2560	0	80
1041	18	0	640	0	160
1044	14	0	10240	0	40
1048	16	0	640	0	40
1053	–	0	10240	0	40
1064	12	0	640	0	40
1069	13	0	160	0	40
1077	13	0	640	0	40
1078	6	0	160	0	40
1081	8	0	5120	0	80
1082	13	0	2560	0	160
1085	23	0	2560	0	40
1089	13	80	640	160	1280
1093	7	0	1280	0	80
1094	4	0	640	0	640
1096	7	0	320	0	160
1097	7	0	320	0	160
1118	13	0	640	0	80
1147	13	0	5120	0	80
1150	5	0	160	0	320

(b)

ID	Interval (Days)	*R. japonica*		*R. typhi*	
		Acute	Convalescent	Acute	Convalescent
1002	10	160	5120	40	2560
1017	16	0	1280	0	40
1019	18	0	160	0	160
1027	22	0	2560	0	640
1029	7	80	1280	80	1280
1036	10	160	1280	0	40
1040	20	0	1280	0	1280
1048	16	40	2560	0	2560
1064	12	0	160	0	1280
1065	20	0	10240	40	20480
1066	15	0	5120	320	10240
1069	13	0	10240	0	40960
1071	14	40	640	0	160
1078	6	0	640	0	80
1082	13	0	2560	80	2560
1089	13	160	20480	640	40960
1094	4	40	2560	40	10240
1095	10	0	640	40	5120
1098	14	0	2560	0	5120
1106	13	0	1280	0	2560
1112	8	0	640	0	640
1113	27	0	80	0	80
1117	4	0	80	40	80
1126	17	640	2560	640	2560
1135	14	0	160	0	40
1148	9	0	1280	0	2560
1150	5	0	160	0	640

–, No data. Ig, immunoglobulin.

In cases with non-specific rickettsial symptoms, the cross-reaction results highlight other possibilities, namely co-infection with *R. japonica* and *R. typhi*, true *R. japonica* infection cross-reacting against *R. typhi* among patients with previous *R. typhi* infection, or true *R. typhi* infection cross-reacting against *R. japonica*. Specifically, in this study, eight indeterminate cases could be attributed to the first two possibilities, although they met the clinical and serological features of JSF.

Declaration of competing interest

The authors have no conflict of interest to declare.

CRediT authorship contribution statement

Tetsuro Aita: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Eiichiro Sando:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Funding acquisition, Supervision, Writing – original draft. **Shungo Katoh:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft. **Sugihiro Hamaguchi:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft. **Hiromi Fujita:** Investigation, Methodology. **Noriaki Kurita:** Data curation, Formal analysis, Supervision, Writing – original draft.

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Ethical approval

The study was approved by Institutional Review Board at Fukushima Medical University.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.03.012.

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