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EACTIVITY BETWEEN RICKETTSIAE AND WEIL-FELIX ANTIGENS AGAINST SERA OF RICKETTSIAL DISEASE PATIENTS

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mary. - Of the sera which were positive to *Rickettsia tsutsuga-ni* by indirect immunoperoxidase test, approximately 80 % sera positive to a *Proteus* OXK antigen by Weil-Felix test at 10 or e days after the onset of fever, while only 10 % sera were posi- within 9 days from the onset of fever. In ELISA using the OXK gen, almost all of the paired sera of tsutsugamushi disease (TD) nts increased on the IgM antibody titres with the rise of their s by Weil-Felix test, whereas the IgG antibody titres of these were unrelated with the titres of Weil-Felix test. We suspect the reactivity of TD patients sera to the OXK antigen in Weil- ix test was derived from the reactivity of the IgM antibody nst the OXK antigen common with *R. tsutsugamushi.* The nt sera infected with a Japanese isolate of spotted fever group ttsia (SFGR) cross-reacted with the Thai Tick Typhus (TTT) n of SFGR by indirect immunoperoxidase test. In Weil-Felix the reactivity of these sera to OX2 antigen were higher than to OX19 antigen, like the sera infected with other SFGR, pt of *R. rickettsii.* These sera also reacted with TTT and OX2 gens by ELISA. The titres of IgM antibody against OX2 antigen ne sera in ELISA were in parallel with the titres of the sera nst OX2 antigen in Weil-Felix test, but not the titres of IgG body. We suggest that the reactivity of the patient sera infected SFGR to OX2 antigen of Weil-Felix test is dependent on the antibody.

words: Weil-Felix test; indirect immunoperoxidase test; Rickettsia ugamushi; spotted fever group rickettsia; immunoblot

Introduction

Some strains of *Proteus* have been used as antigens in the Weil-Felix (WF) test for the diagnosis of rickettsial diseases (Vinson, 1976). Whole cells (WC) of *P. vulgaris* strain OX2 react strongly with the sera from persons infected with spotted fever group rickettsia (SFGR), except with Rocky Mountain spotted fever (RMSF), while the WC of *P. vulgaris* strain OX19 reacts with sera from persons infected with the typhus group rickettsia as well as with RMSF rickettsia. The WC of *P. mirabilis* strains OXK reacts only with sera from persons infected with *Rickettsia tsutsugamushi.* In this study we tried to define the common antigens between *Proteus* and rickettsiae using indirect immunoperoxidase (IP) test, WF test, ELISA, and immunoblotting.

Materials and Methods

Bacteria and growth conditions. Proteus bacteria were grown in liquid medium at 37 °C for 16 hr at shaking (Mizushiri *et al.,* 1990). After cultivation, the bacteria were killed by adding phenol to a final concentration of 0.1 %. *R. tsutsugamushi* was propagated in cultured L cells as described (Suto, 1985). The SFGR strain Thai tick typhus TT-118 (TTT) and the Japanese isolate of SFGR (strain Katayama) were propagated in cultured green monkey kidney cells (Suto, 1985).

Immunological assays. The sera of patients with rickettsial diseases were analysed by IP test, WF test, and ELISA (for detaile see Amano *et al.,* 1990).

SDS-PAGE and immunoblotting. SDS-PAGE was performed as described (Laemmli, 1970) at 12.5 % gel concentration. The gels were stained with silver according to the method of Oakley *et al.* (1980). Immunoblotting was accomplished by a modification of the technique of Towbin *et al.* (1979).

Results and Discussion

Reactivity of sera from tsutsugamushi disease patients by immunological tests

Antisera of tsutsugamushi disease patients were collected from Akita prefecture of Japan in 1989. The sera of 28 patients including paired sera of 22 patients were anlysed by the immunological tests. Among sera that were positive to *R. tsutsugamushi* by IP test, approximately 80 % sera were positive to *Proteus* OXK antigen by WF test at 10 or more days after the onset of fever, while only 10 % sera were positive within 9 days from the onset of fever (data not shown). Table 1 shows the data of IP test, WF test, and ELISA of the selected paired sera from the Tsutsugamushi disease patients. The sera of all patients reacted with three prototype strains, Gilliam, Karp, and Kato of *R. tsutsugamushi* by IP test. Specially, 5 patient's sera reacted strongly with Karp strain, and 1 patient serum (No. 7) with Kato strain. In WF test using OXK whole cells as antigen, titres of the patient sera increased dependent on days after the onset of fever in each patient.

	fever	IP test against *R. tsutsugamushi* strain:						WF test against OXK-WF	ELISA against OXK-WC	
		Gilliam		Karp		Kato			IgG	IgM
		IgG	IgM	IgG	IgM	IgG	IgM			
1	4	<10	<10	<10	<10	<10	<10	<20	3200	200
	8	640	160	640	640	160	160	20	1600	200
	15	2560	1280	5120	5120	2560	1280	80	200	3200
2	4	<10	80	<10	80	<10	80	20	400	3200
	11	160	5120	640	10240	160	2560	>1280	1600	>102400
3	3	<10	<10	<10	<10	<10	<10	<20	1600	3200
	5	<10	20	<10	20	<10	20	20	800	6400
	11	40	1280	640	2560	640	1280	80	1600	6400
	22	160	1280	640	2560	640	2560	160	1600	12800
4	4	10	40	80	160	20	80	20	1600	800
	5	80	640	640	10240	320	2560	40	6400	800
	15	2560	20480	10240	20480	5120	20480	>1280	6400	25600
5	4	<10	20	<10	10	<10	10	20	100	1600
	11	20	1280	160	5120	160	5120	80	<100	6400
6	6	<10	160	10	320	<10	160	<20	6400	3200
	10	320	1280	640	5120	640	5120	80	3200	12800
7	2	<10	<10	<10	<10	<10	<10	<20	400	1600
	7	20	160	20	40	20	160	80	200	6400
	11	20	80	20	20	40	160	80	200	6400
8	3	<10	<10	<10	<10	<10	<10	<20	400	400
	8	640	160	320	160	320	160	20	400	800
	14	5120	1280	5120	1280	5120	1280	320	400	6400

Table 2. Antibody titers of sera from patients with spotted fever diseases measured by IP, WF, and ELISA tests

Patient No.	Days after onset of fever	Antibody titres by:										
		IP test against:				WF test against			ELISA against:			
		Scrub typhus*		TTT					TTT		OX2	
		IgG	IgM	IgG	IgM	OX2	OX19	OXK	IgG	IgM	IgG	IgM
1	11 M**	<10	<10	>10240	<10	160	<20	<20	51200	6400	1600	400
2	138	<10	<10	1280	80	160	20	20	6400	3200	3200	800
3	18	<10	<10	2560	1280	320	80	20	12800	12800	200	3200
	28	<10	<10	2560	160	320	40	20	12800	12800	<100	3200
	83	<10	<10	1280	160	160	40	20	6400	3200	400	400
4	8	<10	<10	40	40	20	<20	20	800	800	800	200
	18	<10	<10	20480	5120	160	80	40	102400	12800	400	1600
5	3 M**	<10	<10	20480	<10	80	<20	<20	>204800	800	12800	400
6	5	<10	<10	40	40	40	20	<20	1600	800	400	400
	9	<10	<10	320	40	80	20	20	1600	1600	800	400
	22	<10	<10	1280	1280	640	40	20	12800	12800	400	6400
	37	<10	<10	1280	320	640	20	20	6400	6400	200	3200
7	12	<10	<10	2560	160	<20	20	20	12800	1600	1600	<100
8	16	20	<10	5120	5120	40	<20	20	122800	800	<100	<100
	35	20	<10	10240	2560	20	<20	<20	25600	800	200	100

*A mixture of *R. tsutsugamushi* strains Gilliam, Karp, and Kato was used for the antigen.
**Months.

We assayed the reactivity of these sera to OXK antigen by ELISA. Almost all of the paired sera increased in the IgM antibody titres with the rise of their titres by WF test, whereas the IgG antibody titres of these sera unrelated with the titres of WF test. Thus, we suspect that the reactivity of Tsutsugamushi disease patients sera against the OXK antigen by WF test is derived from the reactivity of the IgM antibody against the OXK antigen.

Reactivity of sera from patients infected with spotted fever group rickettsia in Japan by immunological tests

Antisera of patients infected with SFGR were collected from Shikoku island of Japan (Mahara, 1987). We analysed 8 patient's sera including 4 paired sera by IP test, WF test, and ELISA (Table 2). These sera cross-reacted with the Thai Tick Typhus (TTT) strain of SFGR by IP test, but not with any strains of *R. tsutsugamushi*. In WF test, the reactivity of these sera to OX2 antigen were higher than that to OX19 antigen. These sera also reacted with strain TTT and OX2 antigens by ELISA. The titres of IgM antibody against TTT antigen in the sera by ELISA seemed to be higher than those by IP test, because of the difference of the techniques used. The titres of IgM antibody against OX2 antigen in the sera by ELISA were in parallel with the titres of the sera against OX2 antigen by WF test, but the titres of IgG antibody were not. The data described above suggest that the reactivity of the sera infected with SFGR to OX2 antigen by WF test is dependent on the reactivity of IgM antibody. According to Cox (1981) in murine typhus infections the *Proteus* agglutinins are IgM class immunoglobulins. However, the nature of antibodies to other rickettsia is unknown.

Finally, we performed immunoblotting to compare the antigenic reactivity of the OX2 antigen in the sera of SFGR patients. The Japanese isolate (strain Katayama) and *Proteus* OX2 whole cells were used as antigens. Among the sera of patients infected with SFGR, IgM antibodies of No. 6 patient serum on 22nd and 37nd days after the onset of fever reacted with the OX2 antigen as well as the Japanese isolate whole cells, but IgM antibodies of the same patient sera on 5th or 9th day after the onset of fever did not. Based on the pattern of immunoblotting, it was recognized that the IgM antibody reacted with the ladder bands of lipopolysaccharide (LPS) contained in OX2 antigen. IgG antibodies of the same patient sera did not show any clear bands. IgG and IgM class antibodies in other sera of the patients infected with SFGR showed many bands reacting with the Japanese isolate antigen. However, these antibodies showed unclear reactivities with the OX2 antigen. The results described above suggest the possibility that OX2-LPS was the antigen in WF reaction.

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