CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, May 1994, p. 318–324 Vol. 1, No. 3
1071-412X/94/$04.00+0

Serological Response of Patients Suffering from Primary and Recrudescent Typhus: Comparison of Complement Fixation Reaction, Weil-Felix Test, Microimmunofluorescence, and Immunoblotting

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Received 8 November 1993/Returned for modification 14 December 1993/Accepted 27 January 1994

Microimmunofluorescence and Western immunoblotting were compared with the classical complement fixation reaction and the Weil-Felix test to study the serological responses of patients to *Rickettsia prowazekii* and both *Proteus vulgaris* OX19 and OX2 during primary and recrudescent typhus infections. The serological response to *R. prowazekii* was found to be similar during primary and recrudescent typhus, and all sera examined contained antibodies to the same *R. prowazekii* cell structures. Immunoglobulin G (IgG) and IgM were found to be the dominant anti-*R. prowazekii* immunoglobulins in all sera tested and were found to be directed against the 100-kDa protein and the lipopolysaccharide. IgA antibodies, when present, were mainly against the 100-kDa protein. For *P. vulgaris*, IgG antibodies recognized the proteins and lipopolysaccharides of both OX19 and OX2 serotypes; IgM antibodies were directed against the *P. vulgaris* OX2 lipopolysaccharide. In addition, donor blood sera, which were negative by microimmunofluorescence, were found to contain IgG immunoglobulins reacting with *R. prowazekii* protein antigens of 135, 60, and 47 kDa by Western immunoblotting.

Typhus fever caused by *Rickettsia prowazekii* is a dangerous epidemic infection which has played a historic role in the outcomes of wars and natural disasters (29). Typhus is considered a potential epidemic danger and continues to be an important global health problem (27, 29).

Typhus occurs in two forms in humans: primary typhus (louse-borne typhus) and recrudescent or relapsing typhus (Brill-Zinsser disease). They present as separate and distinct clinical and epidemiological entities (4, 32). Primary typhus is an epidemic disease which is spread by body lice and manifests itself as a life-threatening acute infection with fever, rash, and encephalitis. The primary infection induces specific antirickettsial antibody production and results in latent *R. prowazekii* persistence in the host, sometimes for the host's lifetime (18, 32). An alteration of the immune control of persistent rickettsial infections causes a recrudescense of latent infection, which is known as recrudescent typhus (31, 32). The biological mechanisms of *R. prowazekii* persistence, relapse, and correlation with immunity are largely unknown. Data concerning the humoral immune response to *R. prowazekii* during primary and recrudescent typhus are limited to the observation of the different avidities of thermolabile complement fixation (CF) antibodies (19), which probably correspond to different levels of immunoglobulin G (IgG) and IgM (20, 21).

The purpose of the work described here was to investigate whether some serological procedures could differentiate primary and recrudescent typhus. Microimmunofluorescense (MIF) and Western immunoblotting (WIB) in comparison with the CF reaction were used to determine which antibodies *R. prowazekii* antigens are directed against. Cross-reactions of sera from patients with typhus with *Proteus vulgaris* OX19 and OX2 and cross-reactions of blood donor sera with *R. prowazekii* were also studied.

MATERIALS AND METHODS

Bacteria. *R. prowazekii* (virulent Breinl strain; Collection of Gamaleya Research Institute of Epidemiology and Microbiology, Moscow, Russia) was cultivated in the yolk sacs of chicken embryos and Vero cell monolayers and were purified from host cell material, respectively, by verografine (SPOFA, Prague, Czech Republic) density gradient centrifugation (1) for WIB or by differential centrifugation (1) for MIF.

P. vulgaris OX19 (OX19) and *P. vulgaris* OX2 (OX2) (Pasteur Institute, Paris, France) were cultivated in Trypticase soy broth (BioMérieux, Marcy l'Etoile, France) at 37°C overnight, washed three times in distilled water by centrifugation, and suspended in water for MIF and WIB. For the Weil-Felix (WF) test, *P. vulgaris* OX19 cells (supplied by State Research Institute on Standardization and Control of Medical Biological Preparations, Moscow, Russia) from one smooth colony were cultivated on semidry meat agar slants (pH 7.0) at 37°C for 24 h, harvested, washed, and suspended in a saline buffer.

Protein concentrations were determined by the Lowry method (22).

Sera. The sera of patients in clinical infectious disease hospital N2 (Moscow, Russia) were collected in 1972, lyophilized, and kept at 4°C in a serum bank (Balayeva N.M., Gamaleya Research Institute of Epidemiology and Microbiology, Moscow, Russia). In the present study, lyophilized sera were restored in a saline buffer.

Sera from blood donors were collected in Marseille in 1988 (Collection of the Centre National de Référence des Rickettsioses, Marseille, France) and were kept frozen at −20°C.

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Description of clinical cases. The ages of the patients ranged from 24 to 70 years, and both sexes were affected. *R. prowazekii* infection was confirmed by the CF test. Diagnosis of either primary typhus or recrudescent typhus was made on anamnestic data (primary typhus between 1936 and 1944 in five patients living in regions where typhus was epidemic) and the present epidemiological situation (outbreak and pediculosis). In all cases, the onset was peracute and was characterized by chills and fever reaching 40°C and greater, severe headaches, and weakness. The state of the patients varied from mild to severe and was associated with prostration, delirium, and sleepiness or, in contrast, excitement, insomnia, and giddiness. A maculopetechial rash from mild to extended was observed on the bodies and extremities of patients, and a petechial-hemorrhagic rash was observed in patients with severe cases of infection. All patients had hyperemia of the face, injected conjunctiva, hypotension, and tachycardia. Cases of infection recognized as primary typhus were more severe, and one patient with primary typhus (patient N6a) died. Diagnosis of murine typhus was excluded epidemiologically by the absence of *Rickettsia typhi* in rats sampled during this period in Moscow and serological data by CF tests with *R. prowazekii* and *R. typhi* antigens. In all patients' sera, the specific titers of anti-*R. prowazekii* antibodies were two and four times greater than the reciprocal titers of anti-*R. typhi* antibodies in parallel reactions with two units of soluble and corpuscular antigens, respectively. Isolation of rickettsiae was not undertaken.

CF test. The CF test was performed with sera inactivated at 56°C for 30 min as described previously (31). Twofold dilutions of decomplemented serum (0.25 ml) were mixed with an equal volume of *R. prowazekii* whole soluble antigen containing 2 U of antigenic activity (Gamaleya Research Institute, Moscow, Russia); this was followed by the addition of 0.25 ml of 2 U of complement (serum from an uninfected guinea pig); the mixture was left at 4°C overnight. Then, 0.5 ml of an activated hemolytic system consisting of hemolytic serum and 3% sheep erythrocytes was added, the mixture was incubated at 37°C for 30 min, and the results were read. The final reaction was estimated within 1 to 2 h, when erythrocytes were completely sedimented. The titers in sera were determined as the highest dilutions of sera at which hemolysis of 50% of the erythrocytes could still be observed. Dilutions with 75 to 100% erythrocyte hemolysis were considered negative.

WF test. The WF test was performed with *P. vulgaris* OX19 agglutinin antigen as described previously (25). Two drops of *P. vulgaris* OX19 antigen were added to 1-ml aliquots of sera at twofold dilutions (1:40 to 1:640) in saline buffer. Sera were incubated at 37°C for 24 h. The endpoint was the highest serum dilution in which distinct clumping could be seen by holding the tube against a dark background.

MIF. MIF was performed by standard procedures (23). Antigens were applied by pen point onto microscope slides, and the slides were air dried and fixed in acetone. Twofold dilutions of sera were prepared in 3% nonfat dry milk in phosphate-buffered saline (PBS), placed onto the antigen slides, and incubated for 30 min at 37°C in a moist chamber. After washing, the slides were treated with specific fluorescein isothiocyanate-conjugated goat anti-human anti-γ chain and anti-μ chain immunoglobulins (BioMérieux) and rabbit anti-human anti-α chain immunoglobulins (Behring, Marburg, Germany) under the same conditions.

To detect IgM and IgA antibodies, absorption of the IgG antibodies was performed (13). Each serum specimen was diluted 1:50 in PBS, mixed with one volume of RF-Absorbent (Behring), and incubated overnight at room temperature. The supernatant obtained after centrifugation of the incubated serum specimen at 12,000 × *g* for 10 min was used for antibody detection. IgG antibody absorption was confirmed for each serum specimen by negative reaction with specific anti-γ chain immunoglobulin conjugate.

SDS-PAGE and WIB. To prepare samples for sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and WIB, whole rickettsial cells were solubilized in the sample buffer of Laemmli (16) and were divided into three portions. One portion was incubated at room temperature for 2 h, another was boiled for 5 min, and the third was used for lipopolysaccharide (LPS) antigen determination and was prepared as follows. Solubilized samples were boiled for 5 min, and proteinase K (Boehringer, Mannheim, Germany) was added to a final concentration of 1 mg/ml. After 1 h of incubation at 56°C, proteinase K was added again and digestion was repeated as described above. The digested samples were boiled for 15 min before loading.

OX19 and OX2 antigens were prepared by solubilization of whole cells in the sample buffer of Laemmli (16) by boiling for 5 min.

Prepared samples were loaded onto 12.5% polyacrylamide gels (10 μg of protein per well) and were separated at 30 mA for 1.5 h by using a Mini-Protein Gel chamber (Bio-Rad, Segrate Milano, Italy) as described previously (12). After migration, a part of the gel was stained with Coomassie R-250 and silver reagent (Bio-Rad) by periodate oxidation (28) to visualize the protein and LPS profiles, respectively.

To perform WIB, polyacrylamide gels were transferred to nitrocellulose membranes (pore size, 0.45 μm; Bio-Rad, Richmond, Calif.) at 50 V for 4 h in 0.025 M Tris base–0.192 M glycine buffer (pH 8.3) containing 20% methanol. After transfer, nonspecific sites on the nitrocellulose membrane were blocked in 5% nonfat dry milk prepared on TTBS buffer (0.02 M Tris-HCl [pH 7.5], 0.5 M NaCl, 0.05% Tween 80) overnight at room temperature, washed in TTBS buffer, and incubated with serum at 37°C for 2 h. For detection of IgG antibodies to *R. prowazekii*, each serum specimen was diluted 1:500 in 3% nonfat dry milk in TTBS buffer. For detection of IgM and IgA antibodies, absorbed sera were used at a final dilution of 1:200. For the OX19 and OX2 antigens, absorbed and nonabsorbed sera were diluted 1:200. Unfixed antibodies were removed by washing for 5 min in distilled water, two 10-min washes in TTBS buffer, and two 10-min washes in TBS buffer (0.02 M Tris-HCl [pH 7.5], 0.5 M NaCl). To detect the specific antibodies, peroxidase-conjugated goat anti-human immunoglobulins (anti-γ and anti-μ chain diluted 1:100 and anti-α chain diluted 1:200 [Diagnostics Pasteur, Marnes-la-Coquette, France] in 3% nonfat dry milk in TTBS buffer) were reacted with the membrane for 1.5 h at room temperature. After washing, bound enzyme was detected by reaction with substrate solution containing 0.015% 4-chloro-1-naphthol (Sigma, St. Louis, Mo.), 0.015% hydrogen peroxide, and 16.7% methanol in TBS buffer. The developed membranes were washed in water, dried between filter papers, and photographed.

Periodate oxidation was used to determine the specific reactions of carbohydrate and protein rickettsial antigens by WIB (30). Antigen transfer membranes were blocked in 0.2% Tween 80 in TBS buffer overnight and were rinsed briefly with 0.05 M sodium acetate buffer (pH 4.5). Control membrane strips were then incubated in this buffer for 1 h, and experimental strips were exposed to 0.04 M sodium periodate in the buffer (pH 4.5) described above for 1 h in the dark at room temperature. Control and experimental strips were then rinsed with the same buffer and were incubated with 1% glycine in PBS for 30 min at room temperature. After three additional 10-min washes with TTBS, membranes were incubated with

TABLE 1. Detection of antibodies to *R. prowazekii* and *P. vulgaris* OX19 and OX2 in sera from patients with primary and recrudescent typhus[a]

Patient	Age (yrs)	Day after onset of fever	CF test result	MIF							WF test	Anti-*R. prowazekii* antibodies in WIB								
				R. prowazekii			*P. vulgaris* OX19		*P. vulgaris* OX2			IgG			IgM			IgA		
												SPA		LPS	SPA		LPS	SPA		LPS
				IgG	IgM	IgA	IgG	IgM	IgG	IgM		TS	TL		TS	TL		TS	TL	
1a	35	9	40	25,600	100	—[b]	—	—	—	—	—	+	+	+	−	+	+	−	−	−
		14	640	102,400	100	—	100	—	100	—	—	+	+	+	+	+	+	−	−	−
		20	640	102,400	200	—	100	—	100	—	—	+	+	+	+	+	+	−	−	−
2a	38	17	1,280	204,800	400	100	400	400	100	—	160	+	+	+	+	+	+	−	+	−
		28	1,280	102,400	100	100	200	100	200	100	80	+	+	+	−	−	+	−	+	−
3a	31	14	320	102,400	100	—	100	—	100	—	40	−	+	+	+	+	+	−	−	−
		23	640	204,800	100	—	100	—	100	—	40	+	+	+	+	+	+	−	−	+
4a	36	11	160	102,400	400	100	400	—	200	—	320	+	+	+	+	+	+	−	+	−
		19	1,280	102,400	100	100	200	—	100	—	320	+	+	+	−	+	+	−	+	−
5a	33	14	640	102,400	800	—	—	—	—	—	320	−	+	+	−	+	+	−	−	−
6a	48	11	320	51,200	25,600	—	200	—	100	—	320	+	+	+	−	+	+	−	+	−
7a	29	10	320	51,200	3,200	400	200	ND	100	ND	160	+	+	+	+	+	+	+	+	+
8a	51	17	640	102,400	1,600	—	400	—	100	—	320	−	+	+	−	+	+	−	−	−
9a	38	15	640	102,400	400	100	400	—	200	—	160	+	+	+	−	+	+	−	+	−
10a	24	15	640	102,400	100	—	—	100	—	—	40	−	+	+	−	+	+	−	−	−
11b	45	14	1,280	102,400	200	100	—	100	—	—	—	+	+	+	+	+	+	−	+	−
		21	1,280	102,400	100	—	100	100	100	—	—	+	+	+	+	+	+	−	+	−
12b	60	14	1,280	204,800	1,600	800	200	—	100	—	160	+	+	+	+	+	+	−	+	−
		22	1,280	204,800	3,200	800	200	—	100	—	320	+	+	+	+	+	+	−	+	−
		28	1,280	204,800	800	200	200	—	100	100	80	+	+	+	−	+	+	−	+	−
13b	70	14	1,280	102,400	100	100	200	—	100	—	160	−	+	+	−	+	+	−	−	−
14b	62	17	640	51,200	200	—	100	—	200	—	40	+	+	+	−	+	+	−	−	−
15b	68	12	640	102,400	200	800	400	—	200	—	160	+	+	+	−	−	+	−	+	−

[a] CF test, complement fixation test with *R. prowazekii* antigen; WF test, Weil-Felix test with *P. vulgaris* OX19 agglutinin antigen; WIB, Western immunoblotting; SPA, major surface protein antigen of 100 to 135 kDa; TS, thermostable modified form of 135-kDa antigen; TL, thermolabile form of the 100-kDa antigen; LPS, lipopolysaccharide antigen. a and b correspond to clinical diagnoses of primary and recrudescent typhus, respectively; + and −, positive and negative reactions, respectively, with the noted antigen; ND, not determined.

[b] —, negative result.

primary and secondary antibodies by the WIB procedure described above.

RESULTS

CF test. Serum specimens from 10 patients with primary typhus and 5 patients with recrudescent typhus were obtained from days 9 to 28 after the onset of fever and were examined by the CF test. All sera were found to be positive in the CF reaction with 2 U of the *R. prowazekii* antigen (Table 1). Increases in specific antibody titers from 40 to 160 to 640 to 1,280 were found when paired serum specimens from two patients with primary typhus were tested between days 9 and 11 and 19 and 20 of illness (see data for patients 1a and 4a in Table 1). For the sera from patients with recrudescent typhus, titers of specific antibodies were 640 to 1,280 and did not change between days 12 and 28 after the onset of fever, when the sera were sampled.

WF test. Agglutinin antibodies to OX19 were found in sera from patients with primary typhus and in sera from patients with recrudescent typhus. The titers ranged from 40 to 320 but appeared to be slightly higher in the sera from patients with primary typhus (Table 1). Sera from one patient with primary typhus (patient 1a) and one patient with recrudescent typhus (patient 11b) were negative in the WF test.

MIF test. The IgG, IgM, and IgA antibody titers to *R. prowazekii* and OX19 and OX2 detected in the sera of patients with typhus are presented in Table 1. All 23 serum specimens contained IgG and IgM antibodies to *R. prowazekii*. The titers of IgG antibodies (1:25,600 to 1:204,800) were significantly higher than those of IgM antibodies (1:100 to 1:3,200). Only one serum specimen from a patient with primary typhus (patient N6a) was found to have similar IgG (1:51,200) and IgM (1:25,600) antibody titers on day 11 of disease. IgA antibody titers of 1:100 to 1:400 were found in 6 of 15 serum specimens from patients with primary typhus and in 6 of 8 serum specimens from patients with recrudescent typhus.

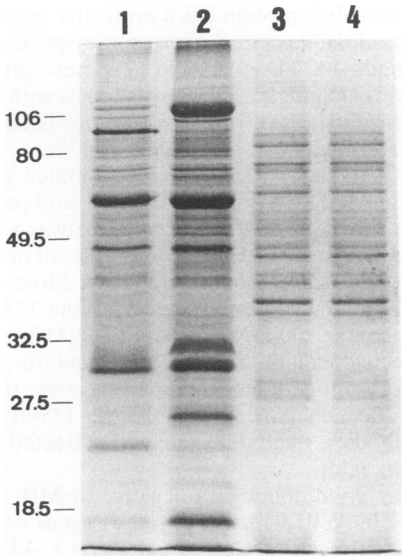

FIG. 1. SDS-PAGE patterns of *R. prowazekii* Breinl, *P. vulgaris* OX19, and *P. vulgaris* OX2 cells. Lane 1, *R. prowazekii* cells solubilized in Laemmli sample buffer at room temperature for 2 h; lane 2, *R. prowazekii* cells boiled in Laemmli sample buffer for 5 min; lane 3, *P. vulgaris* OX19 cells solubilized in Laemmli sample buffer by boiling for 5 min; lane 4, *P. vulgaris* OX2 cells solubilized in Laemmli sample buffer by boiling for 5 min. Protein profiles were stained with Coomassie R-250. The molecular sizes of the protein standards are shown on the left (in kilodaltons).

Anti-OX19 and anti-OX2 IgG antibodies were detected in most of the patient sera tested (Table 1). IgM antibodies were found in six serum specimens: four serum specimens reacted with OX19, one reacted with OX2, and one had a seroconversion to both OX19 and OX2.

SDS-PAGE and WIB. Coomassie R-250-stained, electrophoretically separated *R. prowazekii* whole cells showed major polypeptide bands of 100, 60, 47, 31, 30, 23, and 17 kDa and a number of minor bands when solubilized at room temperature (Fig. 1, lane 1). After boiling, polypeptides of 100, 31, and 23 kDa were heat modified and were revealed as polypeptides of 135, 32, and 26 kDa (Fig. 1, lane 2). The silver-stained LPS profile consisted of a number of periodically repeated bands from 17 to 50 kDa (data not shown).

OX19 and OX2 protein profiles were similar to each other and showed major polypeptide bands of 95 and 87, 73 and 69, 62, 47 and 45, 42 and 40, 38 and 36, and 29 and 27 kDa (Fig. 1, lanes 3 and 4).

By WIB against *R. prowazekii*, two major reactive zones were revealed when IgG antibodies were detected. All tested sera had IgG antibodies against the 100-kDa polypeptide, and most of them also reacted with its heat-modified form of 135 kDa (Table 1 and Fig. 2). These serum specimens also reacted against several low-molecular-mass antigens. This zone, containing a number of antigens from 17 to 50 kDa, corresponded to the position of the O chains of LPS while in the gel only, because these bands disappeared after periodate oxidation. No protein antigens were found in this zone.

IgM antibodies were detected in all serum specimens tested and revealed strong reactions with LPS. Among all of the serum specimens tested, only one from a patient with recrudescent typhus (patient N15b) had no IgM antibodies to protein antigens. Ten other serum specimens contained IgM antibodies against the 100-kDa protein only, while 12 serum specimens reacted with this polypeptide and its heat-modified form.

Specific antirickettsial IgA antibodies were detected in sera from nine patients and were directed against the 100-kDa protein. Two serum specimens differed strongly from the others. One from a patient with primary typhus sampled on day 23 of disease (patient N9a) had IgA immunoglobulins to LPS only, and the other from a patient with a similar diagnosis on day 10 of disease (patient N7a) reacted with 135- and 100-kDa polypeptides and LPS antigens (Fig. 2b).

Paired serum specimens from some patients were compared, and an increase in their reactivities in the course of disease was found. In the serum from a patient with primary typhus (patient N1), development of IgG antibodies to both protein and LPS antigens and IgM antibodies to LPS antigen was observed (Fig. 2a). For another serum specimen from a patient with recrudescent typhus, anti-100-kDa polypeptide antibodies were found at the same level, while anti-LPS antibodies increased (Fig. 3).

Sera from patients with primary typhus and recrudescent typhus were not differentiated by this method and contained IgG, IgM, and IgA antibodies to the same *R. prowazekii* cell structures (Fig. 2 and 3).

When OX19 and OX2 whole-cell antigens were used, sera from patients with typhus reacted with a large number of bands, including polypeptides of 100 to 106, 90 and 80, 64 and 55, 42 and 39, 31 and 28, and 15 kDa and LPS components. Mainly IgG antibodies cross-reacted with both OX19 and OX2 antigens. IgM antibodies from the tested sera reacted only with the LPS of OX2 and not with that of OX19. These positive reactions were found in three of six serum specimens from patients with primary typhus and in three of four serum specimens from patients with recrudescent typhus (Fig. 4).

Randomly chosen sera from 15 blood donors (negative by MIF) were tested with *R. prowazekii* by WIB with anti-γ chain immunoglobulins. Of the tested serum specimens, seven were found to be positive at a 1:100 dilution and reacted with *R. prowazekii* polypeptides of 135, 60, and 47 kDa when antigen was solubilized either at 100°C or at room temperature. When a 1:500 dilution was used, IgG antibodies were not detected in any of the serum specimens tested (Fig. 5).

DISCUSSION

Epidemic and recrudescent typhus infections represent primary and secondary infections, respectively, by the same etiological agent, *R. prowazekii*, separated only by a period of latent rickettsial persistence in the host (4, 32). Traditionally, the immune responses during these two infections are described as primary and secondary reactions to *R. prowazekii* (31) because of differences in 19S (IgM) and 7S (IgG) γ-chain globulins which were found by immunoelectrophoresis (20) and MIF (21) in the sera of patients suffering from these two diseases. Cross-reactions with *Proteus* antigens in patients with primary typhus but not in patients with recrudescent typhus is another characteristic serological response to *R. prowazekii* in humans (5, 17, 32).

In the present study, WIB and MIF together with the CF reaction and the WF test were used to characterize and compare the serological responses of patients suffering from primary and recrudescent typhus infections.

In previous studies, proteins of 100 to 135, 60, 30, and 29 kDa and LPS were found to be the main antigens of *R. prowazekii* detected in the sera of experimentally infected animals (3, 9, 12). A major surface protein antigen found at 100 to 135 kDa is a thermomodified antigen (6, 12). The

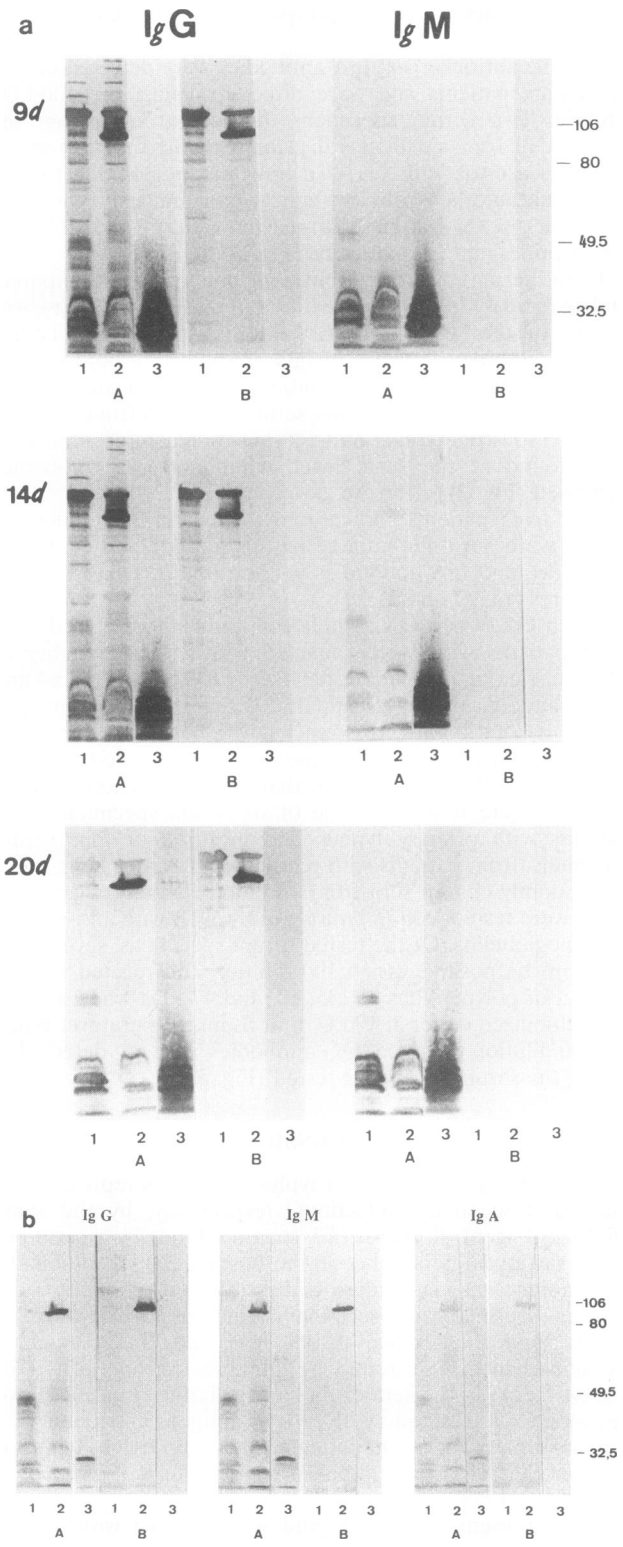

FIG. 2. Reaction by WIB of sera from patients with typhus with *R. prowazekii* antigen. Reactions of IgG (dilution 1:500), IgM (dilution 1:200), and IgA (dilution 1:200) antibodies are shown. Sera were sampled from patient N1a with primary typhus on days 9, 14, and 20 after the onset of fever (a) and from patient N7a with primary typhus on day 10 (b). (A) Untreated control membranes; (B) membranes subjected to periodate oxidation before reaction with serum. Lane 1, whole cells of *R. prowazekii* boiled in Laemmli sample buffer for 5 min; lane 2, whole cells of *R. prowazekii* solubilized in Laemmli sample buffer at room temperature for 2 h; lane 3, LPS antigen of *R. prowazekii*. The molecular sizes of the protein standards are shown on the right (in kilodaltons).

100-kDa thermolabile protein has a protective activity (8), and most of its thermolabile epitopes are species specific (6, 9). The thermomodified 135-kDa protein exposes group-specific epitopes. LPS is an antigen which cross-reacts with the spotted fever group rickettsiae, as well as with other bacteria such as *Proteus* spp. and *Legionella bozemanii* (26).

When the WIB assay was used, all examined patient sera reacted with the 100-kDa polypeptide, LPS, and probably with carbohydrate moieties of glycoproteins between 17 and 50 kDa, including 30- and 31-kDa glycoproteins, all of which were recognized by IgG class immunoglobulins. Most of the sera also had IgG antibodies that reacted with the 135-kDa heat-modified form of the 100-kDa polypeptide. IgM immunoglobulins were directed against the same cell structures, but mainly against the LPS antigen, less frequently against the 100-kDa polypeptide, and only partially against the 135-kDa polypeptide. IgA antibodies, when detected, were directed against the 100-kDa polypeptide.

Some of the blood donor sera negative by MIF were found to be positive by WIB with rickettsial antigens and had IgG antibodies which reacted with 135-, 60-, and 47-kDa polypeptides. The reason for these reactions with the 135- and 47-kDa polypeptides is unknown. Reactions against the 60-kDa protein may be a result of its common structure and immunological properties in eucaryotic and procaryotic organisms (10).

It was found previously that the CF test, MIF, and the microagglutination test gave similar results for the detection of specific anti-*R. prowazekii* antibodies (21). Our data obtained by WIB assay of sera from patients with typhus fever were in good correlation with the serologic tests that were performed, MIF and CF. The dynamics of specific CF antibodies were identical to those recognized in the MIF test. Both methods could detect an increase in the titers of specific antibodies when paired sera from patients with primary typhus were tested. For all other sera, data obtained by either CF or MIF did not reveal specific dynamic changes during the course of disease and were not different in the two groups of patients. All sera were found to have specific IgG immunoglobulins at levels significantly greater than those of IgM. Immunoglobulins of the IgA class were detected in only 50% of the patients. This is the first report of the detection of IgA antibodies during typhus infection.

Sera from patients with typhus were not differentiated by cross-reactivity with *Proteus* antigens. WF test results were mainly confirmed by MIF with a specific rickettsial antigen and the OX19 antigen. Some discrepancies were observed between the WF test and MIF, which were not unexpected, because it has already been shown that extensive differences exist in the results of these two tests for the serodiagnosis of Rocky Mountain spotted fever (14).

Thus, antibodies to *R. prowazekii* in sera from patients with primary typhus and recrudescent typhus are mainly of the IgG and IgM classes and are directed against the 100-kDa polypeptide and LPS. We did not find differences in the specific IgG, IgM, and IgA antibody responses to *R. prowazekii* or in antibodies which cross-react with *Proteus* species during the course of these two different forms of typhus fever and between them. These results are contrary to the commonly accepted view that there are no specific antirickettsial IgM

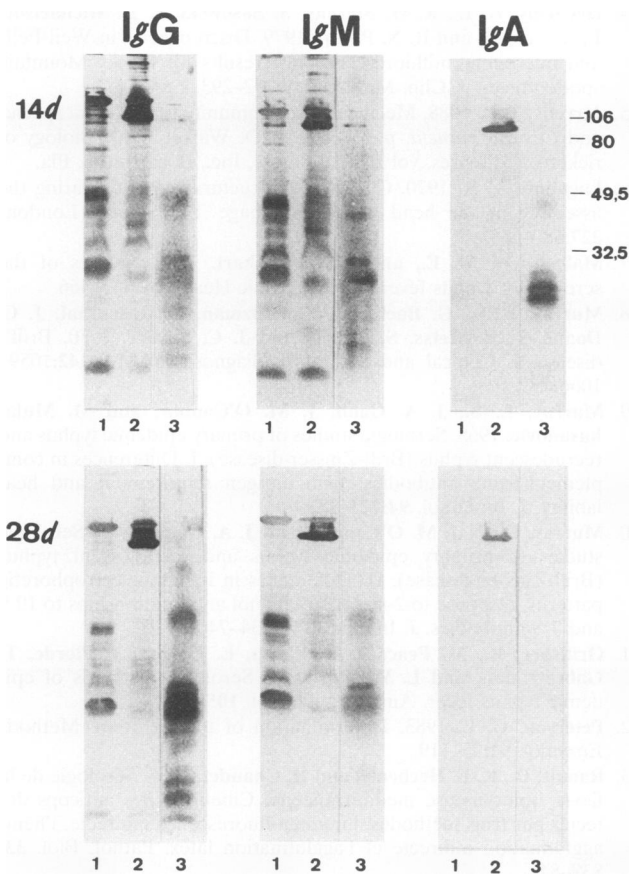

FIG. 3. Reaction by WIB of sera from patients with recrudescent typhus with *R. prowazekii* antigen. Sera from patient N12b were sampled on days 14 and 28 after the onset of fever. The reaction with untreated antigens is shown. The lanes are as described in the legend to Fig. 2.

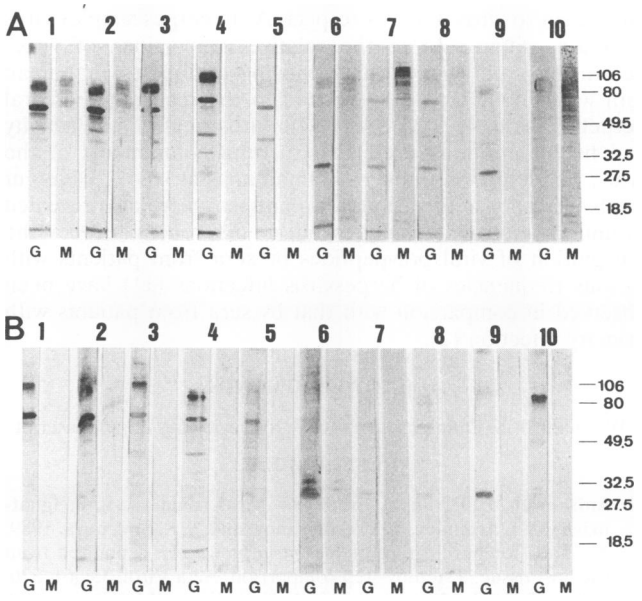

FIG. 4. Reaction of sera from patients with typhus with *P. vulgaris* OX2 (A) and *P. vulgaris* OX19 (B) whole-cell antigens. Sera were diluted 1:200 for the detection of IgG (G) and IgM (M) antibodies. Lanes 1 and 2, sera from a patient with primary typhus (patient N2a) on days 17 and 28 after the onset of fever; lanes 3 and 4, sera from a patient with primary typhus (patient N4a) on days 11 and 19 after the onset of fever; lane 5, serum from a patient with primary typhus (patient N8a) on day 17 after the onset of fever; lane 6, serum from a patient with primary typhus (patient N9a) on day 15 after the onset of fever; lanes 7 and 8, sera from a patient with recrudescent typhus (patient N12b) on days 14 and 28 after the onset of fever; lane 9, serum from a patient with recrudescent typhus (patient N13b) on day 14 after the onset of fever; lane 10, serum from a patient with recrudescent typhus (patient N15b) on day 12 after the onset of fever. The molecular sizes of standard proteins are shown on the right (in kilodaltons).

antibodies and nonspecific anti-*P. vulgaris* antibodies in sera from patients with recrudescent typhus (19–21). However, it is not surprising that sera collected during the course of primary and secondary typhus infections have similar immunoglobulin compositions and react with the same antigenic components of *R. prowazekii*. The immunologic response to the second encounter with the *R. prowazekii* antigen is largely determined by the outcome of the first antigenic challenge. T-cell-dependent antigens such as the 100-kDa protein (6) induce IgG immune responses which determine immunological memory and result in an accelerated response after a secondary exposure to the antigen (15). In contrast, LPS is made up of T-cell-independent antigens and induces the production of short-lived IgM antibodies (15). It is probable that the high level of IgM antibodies in sera from patients with recrudescent typhus might be explained as a new stimulation to the immune system after its second challenge with rickettsiae or may reflect some aspect of the immune response caused by rickettsial persistence. Because LPS is a cross-reacting antigen between *R. prowazekii* and *P. vulgaris* (5, 26), the new production of anti-LPS antibodies might explain our findings of positive WF test reactions at low but diagnostic titers (1:160 to 1:320) in several serum specimens from patients with recrudescent typhus. Positive WF test reactions with similar and higher titers have previously been described by several physicians in patients with recrudescent typhus diagnosed by clinical signs and serology (18).

Similar observations were made concerning Lyme disease and a latent virus infection. The appearance of a new IgM response to *Borrelia burgdorferi* was shown in the chronic arthritic stage of Lyme disease (7). These data suggest a new immune response after the persistence of the Lyme disease agent.

Latent virus infection is characterized by a relapse after the continuous persistence of the agent, and *R. prowazekii* is

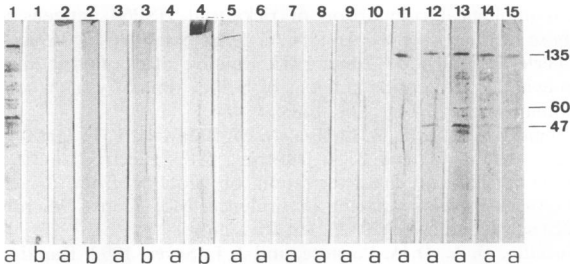

FIG. 5. WIB of blood donor sera with *R. prowazekii* antigens solubilized at room temperature. Numbers on the top correspond to different sera diluted 1:100 (a) and 1:500 (b). The molecular sizes of the reacting antigens are indicated in kilodaltons.

equivalent to viruses in this respect. As in herpes simplex virus infection, differences in the relative proportions of virus-neutralizing and virus-nonneutralizing antibodies in human sera were found and were related to the recurrence of oral herpetic lesions (11, 24). Sera with virus neutralizing activity had higher antibody titers to structural components of the virus, as determined by radioimmunoassay (24). Different levels of response to various viral antigens were also revealed by immunoblotting techniques, but no differences between the recognition of viral polypeptides by sera from patients with various frequencies of herpesvirus infections (11) have been observed in comparison with that by sera from patients with primary infection (2).

ACKNOWLEDGMENTS

We thank J. S. Dumler and P. Kelly for reviewing the manuscript.

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