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# Microimmunofluorescence Test for the Serological Study of Rocky Mountain Spotted Fever and Typhus

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A microimmunofluorescence test was used to study antibody responses to various spotted fever group and typhus group rickettsiae during Rocky Mountain spotted fever (RMSF) and epidemic typhus (ET). Patients with RMSF reacted most strongly to *Rickettsia rickettsii*; those with ET reacted predominantly to *R. prowazekii*. The degree of cross-reaction to other rickettsial strains varied from patient to patient, but a particular pattern of cross-reaction was consistently observed in serial sera from the same patient. Fresh isolates from three Montana RMSF cases were indistinguishable from each other and from strain R of *R. rickettsii* used as a standard antigen in all tests. Immunoglobulin M (IgM) antibodies were usually present in high titer in early-convalescent-phase sera from RMSF, as well as ET, patients. After RMSF, IgM antibodies persisted for a few months and, in one instance, for as long as 10 months. IgM responses to laboratory-acquired infections were infrequent in persons previously vaccinated with antigens related to the infecting strain. Previous antigenic conditioning from infection or vaccination may have accounted partly for the apparent lack of IgM response in a few study participants.

Indirect immunofluorescence (IF) is not new to serological study of rickettsial infections. Goldwasser and Shepard (7) first demonstrated its potential value for the study of typhus, and Bozeman and Elisberg (3) found it helpful in the investigation of scrub typhus, in which antigenically diverse strains complicate serological responses. Hazard et al. (10) used IF in conjunction with complement fixation (CF) tests to identify cases of Rocky Mountain spotted fever (RMSF) in New England, and Gutman et al. (8) reported its use to confirm diagnoses of tick-borne typhus in Israel. However, IF has not been used extensively in the investigation of RMSF, nor has a practicable procedure been described that is applicable to routine serodiagnosis of rickettsial infections.

Five years ago, Wang (18) developed a micro-IF method that is widely recognized as a simple, reliable procedure for immunological classification of trachoma-inclusion conjunctivitis and lymphogranuloma venereum chlamydiae (19). Since then, it has been used increasingly to characterize serological responses to trachoma-inclusion conjunctivitis and lymphogranuloma venereum infections in humans (5, 9, 16, 20). The test is sensitive, reproducible, and often measures antibodies to species-specific antigens of infecting strains.

On the supposition that micro-IF might also be efficacious for the study of human rickettsial infections, particularly RMSF, a resurging health problem in the United States, we evaluated the procedure in tests on sera from epidemic typhus (ET) and RMSF patients. This report describes the method and presents general observations on IF antibody responses to RMSF and ET. Use of the procedure relative to other serological tests in serodiagnosis of RMSF and typhus will be reported elsewhere.

## MATERIALS AND METHODS

**Rickettsial strains.** Six strains were used for the preparation of antigens routinely used in all tests (standard antigens). Three were from the spotted fever group and three were from the typhus group. They included: *Rickettsia rickettsii*, strain R, 53 chicken embryo passages (EP), a virulent strain isolated from *Dermacentor andersoni* ticks in Montana in 1945 (2); *R. akari*, strain 29 of American rickettsialpox, 17 EP; *R. conorii*, Malish strain of South African tick-bite fever, 12 EP (2); *R. prowazekii*, strain Breinl, 164 EP; *R. typhi*, strain Wilmington, 14 EP; *R. canada*, strain 2678, 11 EP (12).

Other spotted fever group strains were occasionally included in a given series of tests. These rickettsiae were isolated from ticks from various areas of the United States but have not yet been incriminated as agents of human disease. They included the *Haemaphysalis leporispalustris* strain of *R. rickettsii* (15), the maculatum strain of *R. parkeri* (14), the M/5-6B strain of *R. montana* (1), and a recently

described rickettsia isolated from *Rhipicephalus sanguineus* ticks in Mississippi (4).

**Antigens.** Standard antigens were rickettsiae that were cultivated in chicken embryo yolk sacs, inactivated with 0.2% formalin, and purified by the method of Ormsbee and Peacock (submitted for publication). Stock suspensions of purified rickettsiae were standardized in normal saline containing 1:10,000 merthiolate solution (Eli Lilly & Co., Indianapolis, Ind.) to given 200 $\mu$g of rickettsiae/ml. All stock suspensions were prepared between April 1971 and December 1972 and held at 4 C. Working antigens were prepared as needed by adding 0.5 ml of stock suspension to 0.5 ml of 0.01 M phosphate-buffered saline, pH 7.4, containing 5% uninfected (normal) yolk sac and held at 4 C.

Several experiments were performed to determine the effects of inactivation, purification, and storage on reactivity of antigens. For this purpose, 5% crude yolk sac preparations containing viable rickettsiae of the same strains were compared with working preparations of standard antigens in micro-IF tests of sera from convalescent RMSF cases and antisera prepared against the same suspensions of active rickettsiae in mice. Homologous antibody titers were usually the same, and never more than twofold higher, against active rickettsiae than against inactive rickettsiae in working preparations of standard antigens used regularly for as long as 4 months. Furthermore, patterns of reactivity of the two kinds of antigens with heterologous sera were similar. These observations, plus the fact that stock suspensions of standard antigens had been prepared 3 to 4 years earlier, indicated that the suspensions were remarkably stable when stored under the conditions described and that formalin had had little or no effect on reactivity.

**Micro-IF tests.** The procedure for micro-IF tests was that of Wang (18). The important features of the method are that small quantities of reagents are used, as many as nine dilutions of one serum (or single dilutions of nine sera) can be tested on one slide, and the same drop of diluted serum is reacted against many (usually nine) antigens simultaneously. Rickettsial antigens were applied by dip-pen point (C. Howard Hunt Pen Co., Camden, N.J.) to microscope slides over premarked templates in nine sets of dots, nine antigens per set. Six were standard antigens; the other three were various preparations described above, introduced into a particular series of tests for comparison. After application to slides, antigens were dried for 30 min and fixed in acetone at room temperature for 10 min. Serial twofold dilutions of serum in phosphate-buffered saline containing 10% normal yolk sac to reduce background fluorescence were then placed on antigen sets with a 3-mm bacteriological loop. An appropriate positive or negative control serum was added to the antigen sets in the upper left corner of each slide. The slides were incubated in moist chambers for 30 min at 37 C and washed in two changes of phosphate-buffered saline for 5 min each. After drying, antigen sets were overlaid with loopfuls of fluorescein isothiocyanate-labeled goat anti-human globulin (Baltimore Biological Laboratories, Cockeysville, Md.) or conjugated rabbit anti-human immunoglobulin M (IgM), $F_c$ specific (Industrial Biological Laboratories, Inc., Rockville, Md.). The anti-Ig conjugate detected antibodies in both IgM and IgG classes. This was determined after absorption of the conjugate with purified IgG or IgM in tests of typhus antisera with and without IgM antibodies. Evidence for specificity of the anti-IgM conjugate was described elsewhere (16). After conjugate was added, the slides were incubated for 30 min at 37 C, washed for 5 min each in two changes of phosphate-buffered saline, and mounted in buffered glycerol.

A typical test consisted of 20 slides (20 serum titrations) set up in the morning and examined in the afternoon. The slides were read independently by two persons who did not know the identity of a particular serum being examined. End points against each antigen were the highest serum dilutions conferring definite fluorescence to rickettsiae. The lower of the two end point readings was designated as the antibody titer. Although end points were subjectively determined, reproducible results were readily obtained with experience.

**Sera.** About 1,700 sera have been tested since this work began in October 1973. Included were several large groups of paired sera from Ethiopian patients suspected to have ET or louse-borne relapsing fever (Ormsbee, Peacock, Plorde, Philip, and Casper, submitted for publication); sera submitted to the Rocky Mountain Laboratory in 1973 and 1974 by physicians in northwestern states from patients with tick-associated illnesses, many of which were considered to be RMSF or Colorado tick fever; sera from Mississippi patients with tick-associated illnesses in 1973; and all sera referred to the North Carolina Division of Health Services in 1974 for rickettsial serology (Philip, Casper, Thomas, Anacker, Burgdorfer, MacCormack, and Sexton, submitted for publication). Small groups of sera from various sources and clinical entities were also examined primarily to determine specificity of the test and parameters of reaction. Included among these were stored sera from eight laboratory-acquired rickettsial infections that occurred between 1959 and 1974.

All sera were screened at 1:16 dilutions for rickettsial antibodies, and those reacting were titrated to end point. Most sera were also tested for CF antibodies by the standard procedure used at Rocky Mountain Laboratory (21). Many were also tested for typhus and RMSF antibodies by microagglutination (6) and for spotted fever group antibodies by passive hemagglutination (R. Anacker, submitted for publication).

The results herein are based on all micro-IF reactive sera from the above groups. The precise number of typhus and RMSF cases represented is unknown, and it is not intended in this report to establish criteria for definition of a seropositive case. However, some concept of the numbers of cases involved may be gained from the fact that 59 and 94 patients with paired sera had fourfold or greater rises in micro-IF antibody titers to *R. prowazekii* and *R. rickettsii*, respectively. Most of

these persons also had other serological evidence of infection by these rickettsiae.

## RESULTS

**Description of micro-IF reactions.** Purified rickettsial suspensions were combined with normal yolk sac for three reasons. (i) Innate fluorescence of rickettsiae is obscured by homogeneous, low-background fluorescence of yolk sac (Fig. 1A). When specific binding of antibody occurs, rickettsiae become visible (Fig. 1B, C). End points based on the disappearance of rickettsiae can be sharply determined. (ii) The presence of yolk sac facilitates the location of antigen dots. (iii) Rickettsiae plus yolk sac adhere to slides better than rickettsiae alone.

Specific reactions were characterized by sharp, uniform, and intense staining of rickettsiae in particular antigen plaques. They were particularly evident against *R. rickettsii* in sera from RMSF cases (Fig. 1B, E, F) and against *R. prowazekii* in sera from typhus patients (Fig. 1C). Cross-reactions of varying degree sometimes occurred to antigens both within (Fig. 1E, F) and between (Fig. 1D) the spotted fever and typhus groups. Staining characteristics of cross-reacting antigens varied widely. In some instances, rickettsiae stained specifically but only with low dilutions of serum. In others, staining was erratic; i.e., intensity of fluorescence varied, and organisms were indistinct and appeared granular or fuzzy, as though antibody were bound only to few antigenic determinants or were loosely bound.

**Antibody responses in laboratory-confirmed RMSF.** Organisms characterized as fully virulent *R. rickettsii* were isolated from three clinically typical cases of RMSF from the Bitter Root Valley, Mont. Active rickettsiae in crude yolk sac preparations of the three isolated strains were compared with standard antigens in micro-IF tests of acute and convalescent sera from the patients. The results are shown in Table 1. Only one patient had diagnostic ($\geq$fourfold) rise in CF spotted fever antibodies, but all three showed pronounced responses to spotted fever group antigens by micro-IF. No differences were noted in reactivity among isolates or between the isolates and strain R of *R. rickettsii*.

**Heterotypic antibody responses in RMSF.** To date we have not found sera from tick-associated illnesses in the United States that reacted more specifically to other spotted fever group agents or *R. canada* than to the R strain of *R. rickettsii*. Although all sera from convalescent RMSF patients showed at least some cross-reaction with other spotted fever group antigens, the degree of response was always as high or higher against the R strain. However, patterns of reactivity to heterologous antigens of the spotted fever group varied from patient to patient. Often there were no cross-reacting, typhus group antibodies. When these did occur, titers were usually low and patterns of reactivity to particular typhus group rickettsiae were variable. Variability in reaction patterns seemed to be host-associated rather than a peculiarity of a given serum. Thus, a particular pattern of cross-reaction was consistently observed in serial sera from the same patient.

Some variations in antibody responses in RMSF are shown in Tables 1 and 2. In Table 1, patient 1 had rather broad antibody responses to typhus, as well as spotted fever, group strains as compared with the specific responses to *R. rickettsii* in patients 2 and 3, even though the three isolates from these patients were indistinguishable when tested against the convalescent-phase sera.

In Table 2, patient 1 was unusual for the late appearance of high antibody titers quite specific for *R. rickettsii*. Early-convalescent-phase sera were broadly reactive at low levels to all spotted fever group strains. None of his sera cross-reacted with typhus group antigens.

Patient 2 developed high antibody levels early, particularly directed toward *R. rickettsii*. Although she had modest titers to *R. conorii*, she developed only low titers to *R. akari*. She also had transient antibodies in low titer to *R. canada* but none to other strains of the typhus group. The tendency toward spec-

FIG. 1. *Micro-IF standard test antigens overlaid with 1:16 dilutions of serum and stained with fluorescein-conjugated goat antihuman globulin. ×1,200. (A) Rickettsia prowazekii, left, and R. rickettsii, right, overlaid with serum from a patient with Colorado tick fever. Antibody titers against both antigens were <1:16. (B) R. prowazekii, left, and R. rickettsii, right, overlaid with serum from a patient with RMSF. Antibody titers were <1:16 against R. prowazekii and 1:2,048 against R. rickettsii. (C) R. prowazekii, left, and R. rickettsii, right, overlaid with serum from a patient with ET. Antibody titers were 1:32,768 against R. prowazekii and <1:16 against R. rickettsii. (D) R. prowazekii, left, and R. rickettsii, right, overlaid with serum from a patient with indeterminate rickettsial disease. Antibody titers were 1:4,096 against R. prowazekii and 1:1,024 against R. rickettsii. (E) R. akari, left, and R. rickettsii, right, overlaid with serum from a Mississippi patient with RMSF. Antibody titers were 1:2,048 against both antigens. (F) R. akari, left, and R. rickettsii, right, overlaid with serum from a Montana patient with RMSF. Antibody titers were 1:32 against R. akari and 1:4,096 against R. rickettsii.*

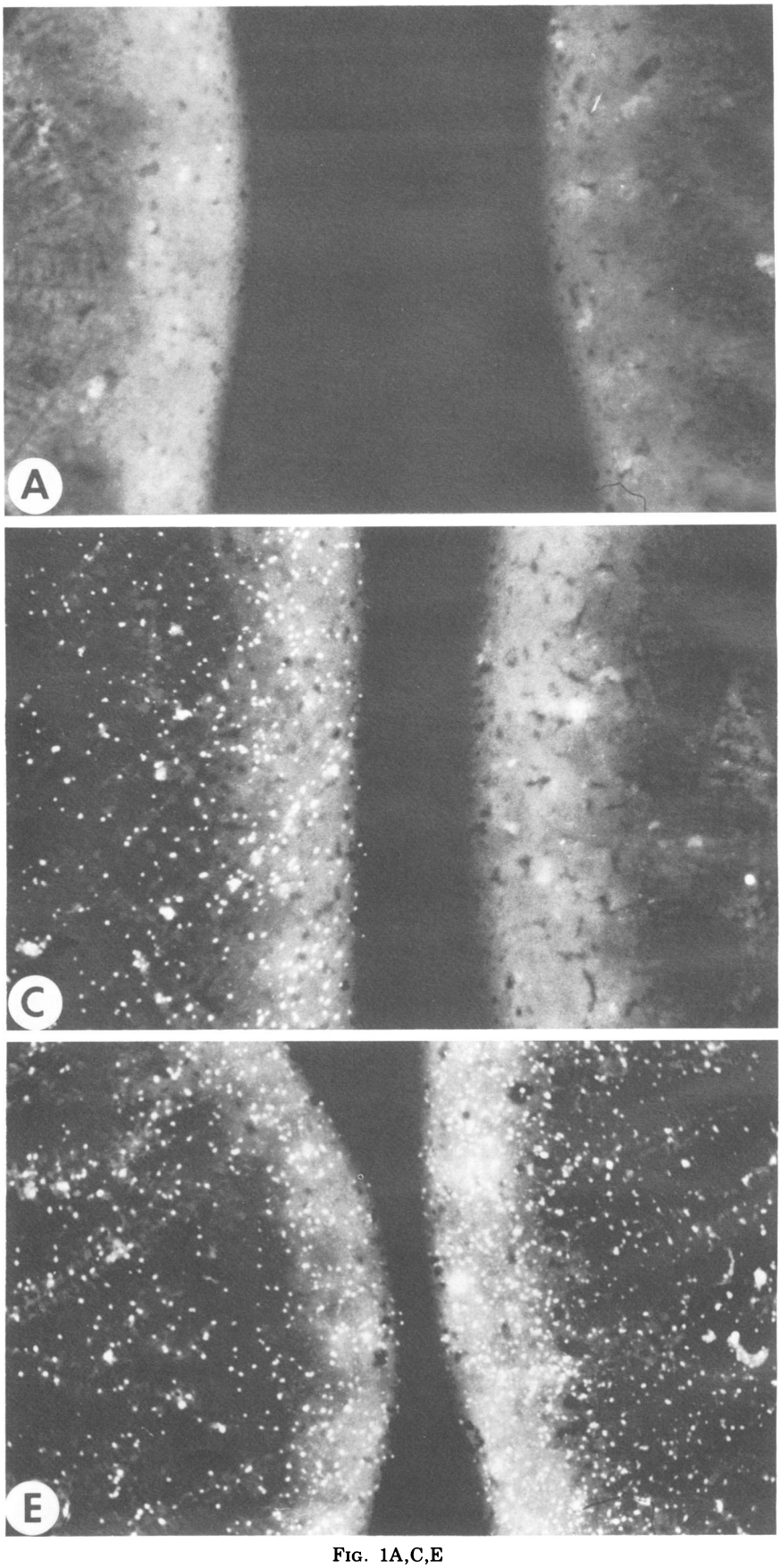


FIG. 1A,C,E

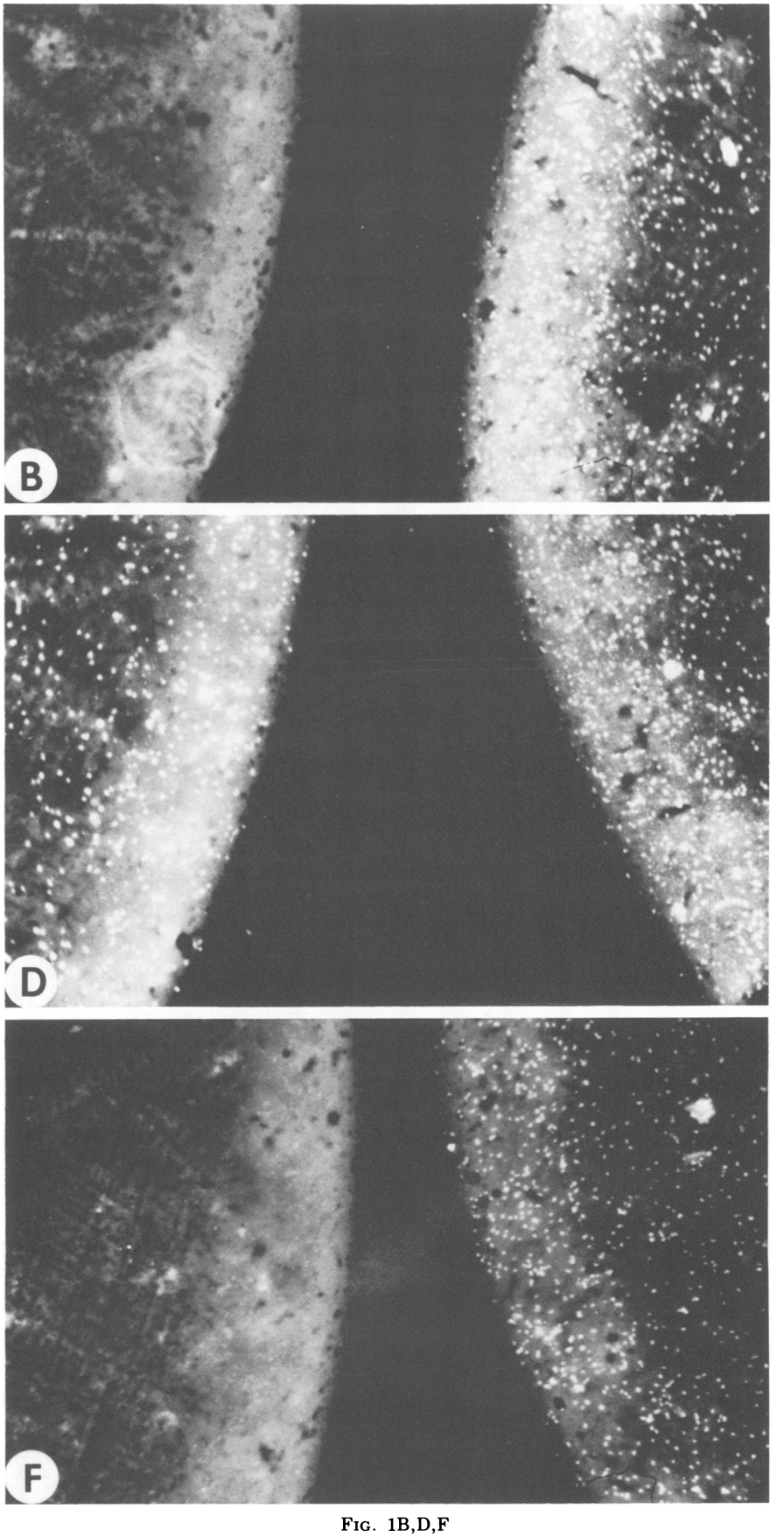


FIG. 1B,D,F

TABLE 1. *Micro-IF serum antibody responses in RMSF patients from whom rickettsiae were isolated*

| Patient | Disease onset | Illness day | CF titer[a] | Micro-IF titers to[b]: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Isolated strains[c] | | | Standard test antigens[d] | | | | | |
| | | | | | | | Spotted fever group | | | Typhus group | | |
| | | | | RS | HH | MW | rick | akar | cono | prow | typh | cana |
| 1. RS male, 5 yr old | 7-13-70 | 4 | 0 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | | 25 | 8 | 8,192 (8,192) | 8,192 (8,192) | 8,192 (8,192) | 4,096 (4,096) | 1,024 (512) | 1,024 (1,024) | 16 (0) | 32 (32) | 128 (128) |
| 2. HH male, 25 yr old | 6-2-73 | 0 | 0 | NT (NT) | NT (NT) | NT (NT) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | NT (NT) | NT (NT) |
| | | 40 | 12 | 128 (64) | 128 (128) | 128 (64) | 64 (64) | 16 (16) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | | 137 | 48 | 8,192 (0) | 8,192 (0) | 8,192 (0) | 8,192 (0) | 32 (16) | 128 (0) | 0 (0) | 0 (0) | 0 (0) |
| 3. MW male, 6 yr old | 5-4-74 | 2 | 0 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | | 24 | 6 | 256 (256) | 256 (256) | 256 (256) | 256 (256) | 32 (32) | 32 (0) | 0 (0) | 0 (0) | 0 (0) |
| | | 51 | 8 | 16 (0) | 32 (0) | 32 (0) | 16 (0) | 16 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |

[a] CF antibody titers to *R. rickettsii*, R strain. 0 = titers < 1:6.
[b] Numbers not in parentheses indicate titers of antibodies undifferentiated as to Ig class. Numbers in parentheses indicate titers of IgM antibody. 0 = titers < 1:16; NT, not tested.
[c] Crude yolk sac preparations (5%) containing viable rickettsiae.
[d] rick, *R. rickettsii*; akar, *R. akari*; cono, *R. conorii*; prow, *R. prowazekii*; typh, *R. typhi*; cana, *R. canada*.

ificity to *R. rickettsii* was frequently, but not exclusively, encountered in RMSF patients from the Rocky Mountain area.

Patient 3 is notable for his unusually pronounced antibody response to several typhus, as well as spotted fever, group antigens. If IgM antibodies to *R. rickettsii* had not been present, serodiagnosis of this case would have been difficult. Previous ET vaccination is unknown.

Patient 4 had broad reactivity in convalescent serum to spotted fever and typhus group strains. Because of his age and IgM response, it is doubtful that he had received typhus vaccine. His reactivity probably represents a primary response to antigens shared by both groups of organisms.

**Antibody responses to typhus.** All sera from convalescent Ethiopian typhus patients reacted most strongly to *R. prowazekii*. Nevertheless, there was uniformly pronounced cross-reaction to *R. typhi* (titers averaging fourfold lower than to *R. prowazekii*) and modest reactions to *R. canada* (titers averaging eightfold lower than to *R. prowazekii*). Although cross-reactions were often also noted against spotted fever group antigens, particularly *R. conorii*, titers and reaction patterns varied, and some patients were devoid of spotted fever group antibodies. Examples of variations in reaction are shown in Table 3.

Patient 85, devoid of antibodies early, subsequently developed high typhus titers, particularly to *R. prowazekii*, but barely detectable cross-reacting, spotted fever group antibodies.

Patient 225 was also devoid of antibodies early, but later he acquired high levels of broadly reacting spotted fever group, as well as typhus group, antibodies.

Patient 162 had equally high titers of antibody to *R. prowazekii*, *R. rickettsii*, and *R. conorii* on day 13 of illness but virtually no reaction to *R. akari*. By day 27, a fourfold rise in titer to *R. prowazekii* had occurred, but titers remained the same against other antigens. This may have been ET in a person who had experienced a spotted fever group infection.

Patient 155 illustrates the extremely high antibody responses to *R. prowazekii* occasionally noted. Homologous antibody responses in ET were generally higher than they were in RMSF.

**IgM antibody responses.** IgM antibodies were detected in 85% of persons with diagnostically significant antibody responses to *R. rickettsii* or *R. prowazekii*. Some IgM responses are shown in Tables 1 to 3. Frequency of homologous IgM antibodies was similar in typhus and

TABLE 2. *Variations in micro-IF serum antibody responses in RMSF patients*

| Patient | Disease onset | Illness day | CF titer[a] | Micro-IF titers to[b]: Spotted fever group rick | akar | cono | Typhus group prow | typh | cana |
|---|---|---|---|---|---|---|---|---|---|
| 1. JD male, 12 yr old | 5-16-74 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | (0) | (0) | (0) | (0) | (0) | (0) |
| | | 15 | 24 | 256 | 256 | 256 | 0 | 0 | 0 |
| | | | | (128) | (128) | (256) | (0) | (0) | (0) |
| | | 32 | 32 | 512 | 256 | 128 | 0 | 0 | 0 |
| | | | | (128) | (64) | (64) | (0) | (0) | (0) |
| | | 111 | 64 | 4,096 | 256 | 256 | 0 | 0 | 0 |
| | | | | (0) | (0) | (0) | (0) | (0) | (0) |
| 2. HC female, 63 yr old | 8-19-73 | 8 | 8 | 256 | 0 | 16 | 0 | 0 | 0 |
| | | | | (32) | (0) | (0) | (0) | (0) | (0) |
| | | 19 | 16 | 8,192 | 128 | 1,024 | 0 | 0 | 128 |
| | | | | (1,024) | (32) | (256) | (0) | (0) | (16) |
| | | 59 | 6 | 8,192 | 128 | 1,024 | 0 | 0 | 16 |
| | | | | (1,024) | (32) | (256) | (0) | (0) | (0) |
| | | 322 | 8 | 1,024 | 0 | 256 | 0 | 0 | 0 |
| | | | | (128) | (0) | (32) | (0) | (0) | (0) |
| 3. RL male, 43 yr old | 8-11-63 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | (0) | (0) | (0) | (0) | (0) | (0) |
| | | 52 | 16 | 2,048 | 64 | 512 | 4,096 | 1,024 | 16 |
| | | | | (64) | (0) | (32) | (0) | (0) | (0) |
| 4. BC male, 4 yr old | 7-17-73 | 10 | 0 | 32 | 32 | 16 | 32 | 0 | 0 |
| | | | | (64) | (64) | (32) | (64) | (16) | (16) |
| | | 22 | 16 | 256 | 128 | 128 | 32 | tr | 16 |
| | | | | (256) | (128) | (32) | (128) | (32) | (64) |

[a] CF antibody titers to *R. rickettsii*, R strain. 0 = titers < 1:6.
[b] See footnotes b and d, Table 1.

TABLE 3. *Variations in micro-IF serum antibody responses in Ethiopian ET patients*

| Patient no. | Disease onset | Illness day | CF titer[a] | Micro-IF titers to[b]: Spotted fever group rick | akar | cono | Typhus group prow | typh | cana |
|---|---|---|---|---|---|---|---|---|---|
| 85 | 4-10-70 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | (0) | (0) | (0) | (0) | (0) | (0) |
| | | 17 | 64 | 32 | 32 | 32 | 32,768 | 16,384 | 4,096 |
| | | | | (16) | (32) | (32) | (8,192) | (2,048) | (512) |
| 225 | 3-26-71 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | (0) | (0) | (0) | (0) | (0) | (0) |
| | | 17 | 256 | 1,024 | 2,048 | 1,024 | 16,384 | 2,048 | 1,024 |
| | | | | (0) | (0) | (0) | (1,024) | (1,024) | (512) |
| 162 | 3-4-70 | 13 | 64 | 2,048 | 32 | 2,048 | 2,048 | 512 | 256 |
| | | | | (0) | (0) | (0) | (2,048) | (512) | (256) |
| | | 20 | 256 | 2,048 | 32 | 2,048 | 4,096 | 512 | 256 |
| | | | | (0) | (0) | (0) | (1,024) | (128) | (64) |
| | | 27 | 128 | 2,048 | 64 | 2,048 | 8,192 | 256 | 256 |
| | | | | (0) | (0) | (0) | (32) | (64) | (32) |
| 155 | 8-23-70 | 15 | 32 | 32 | 64 | 16 | 4,096 | 1,024 | 256 |
| | | | | (0) | (0) | (0) | (0) | (0) | (0) |
| | | 30 | 64 | 64 | 512 | 128 | 65,536 | 8,192 | 4,096 |
| | | | | (0) | (0) | (0) | (0) | (0) | (0) |

[a] CF antibody titers to *R. prowazekii*, Breinl strain. 0 = titers < 1:4.
[b] See footnotes b and d, Table 1.

RMSF patients. In general, IgM responses corresponded to Ig responses in time of appearance of antibody, antibody titers in early-convalescent-phase sera, and spectra of reactivity against heterologous antigens. Although late-convalescent-phase sera were few, there was evidence that IgM, but not Ig, antibodies had begun to decline several months after RMSF (e.g., patient 2, Table 1; patient 1, Table 2). No late-phase sera were obtained from typhus patients.

There were exceptions to these observations. Occasionally in very early-phase sera, low levels of IgM antibody were detected in the absence of Ig reactivity. The anti-IgM conjugate was probably more efficient than the anti-Ig conjugate in detecting IgM antibodies. Titers of IgM antibody were sometimes quite low relative to Ig titers in early-, as well as late-, convalescent-phase sera. Occasionally, fluorescence with the anti-IgM conjugate was less intense at low than at high serum dilutions, as though binding of IgM antibody were being inhibited in the test system, perhaps by more avid IgG antibody. Some RMSF and typhus patients with strong heterologous rickettsial group Ig responses showed no cross-reacting IgM antibodies (e.g., patient 3, Table 2; patients 225 and 162, Table 3). Some of these persons may have had earlier contact with heterologous antigens, either from infection or immunization. Finally, IgM antibody did not always disappear within a few months after infection (e.g., patient 2, Table 2, still had homologous IgM reactivity more than 10 months after RMSF).

About 15% RMSF, as well as typhus, patients showed no IgM response. Sometimes this was observed in the presence of extremely high levels of Ig antibody. Yet, usually absence of IgM reactivity seemed to be characteristic for a particular patient rather than a particular serum. Thus, early-convalescent-phase sera with modest Ig titers from such patients were devoid of IgM reactivity (e.g., patient 155, Table 3).

**Laboratory-acquired RMSF and typhus in vaccinated persons.** Acute- and convalescent-phase sera, stored frozen, were still available from two laboratory-acquired cases of RMSF, five cases of typhus (presumably murine), and one undifferentiated spotted fever group infection. All patients had received RMSF and/or ET vaccine before illness.

Table 4 shows micro-IF responses to illness in five of these patients. Patient 1 acquired RMSF by tick bite in 1959. He had received RMSF vaccine in at least 4 different years; the last dose was given 7 years before illness. Spotted fever group antibodies were detected by CF, but not by micro-IF, on day 1 of illness. On day 10, a specific micro-IF response of Ig antibodies to *R. rickettsii* was demonstrated. IgM reactivity was absent.

Patient 2, an RMSF case, was probably infected by aerosol in 1962, 4 months after receiving two doses of RMSF vaccine. On day 6, CF and low levels of micro-IF antibody against *R. rickettsii* were detected. By day 30, he had high levels of micro-IF antibody quite specific for *R. rickettsii*. An IgM response was never detected.

Patients 3 and 4 were infected simultaneously by aerosol with murine typhus rickettsiae. Patient 3 had had ET vaccine 3 years earlier but no spotted fever vaccine. He developed CF and micro-IF responses specific for typhus group rickettsiae with highest titers directed toward *R. prowazekii*. Typhus antibodies persisted for at least several years. Patient 4 had had both RMSF and typhus vaccines 6 years earlier. He developed high levels of antibodies against typhus rickettsiae, particularly *R. prowazekii*, and also low, broadly reacting antibody titers against spotted fever group rickettsiae. After 2 years, modest titers of typhus antibodies were still demonstrable by micro-IF. IgM antibodies were not detected in either patient.

Patient 5 acquired typhus, probably murine, by aerosol in 1970. He had been vaccinated annually for many years against RMSF and ET, but the last dose of typhus vaccine was received 18 years before illness. Between day 4 and 24, he developed high CF and micro-IF antibody titers against typhus group and moderate titers against spotted fever group rickettsiae. Titers were highest against *R. prowazekii*. Low levels of antibody against both groups of organisms were still present 2 years later. Of particular interest was the fact that this patient also had a transient IgM antibody response against *R. prowazekii* and *R. canada* but not against *R. typhi*.

These observations suggested that antibody response to illness in vaccinated persons was directed particularly toward the related vaccine strain. Heterologous rickettsial group antibody response to illness was sometimes observed in persons who had received heterologous group vaccines. IgM responses to infection were infrequent (two of eight patients) in persons previously sensitized to related antigens.

## DISCUSSION

An advantage of IF over most serological procedures for study of rickettsiae is that antibody interaction with surface antigens of indi-

TABLE 4. *Micro-IF serum antibody responses to laboratory-acquired RMSF and typhus in vaccinated persons*

| Patient, disease, vaccination history[a] | Illness day | CF titer[b] | Micro-IF titers to[c]: | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Spotted fever group | | | Typhus group | | |
| | | | rick | akar | cono | prow | typh | cana |
| 1. GS RMSF 6-1-59 | 1 | 48 | 0 | 0 | 0 | 0 | 0 | 0 |
| Vac: RMSF—1947, 1948, 1949, 1952 | 10 | 128 | 128 | 0 | 0 | 0 | 0 | 0 |
| ET—1942, 1947 | | | | | | | | |
| 2. LP RMSF 9-5-62 | 6 | 128 | 128 | 0 | 0 | 0 | 0 | 0 |
| Vac: RMSF—May 1962 (2 doses) | 14 | 256 | 1,024 | 16 | 32 | 0 | 0 | 32 |
| ET—June 1962 (2 doses) | 30 | 256 | 4,096 | 32 | 128 | 16 | 0 | 16 |
| 3. JC Typhus 12-26-69 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Vac: RMSF—none | 18 | ≥64 | 0 | 0 | 0 | 256 | 64 | 32 |
| ET—1966 | 27 | ≥64 | 0 | 0 | 0 | 256 | 128 | 64 |
| | 1 yr | 16 | 0 | 0 | 0 | 256 | 32 | 16 |
| | 2 yr | 16 | 0 | 0 | 0 | 128 | 32 | 0 |
| 4. KB Typhus 12-26-69 | 8 | 12 | 0 | 0 | 0 | 64 | 16 | 0 |
| Vac: RMSF—1963 | 13 | ≥256 | 64 | 64 | 16 | 4,096 | 1,024 | 256 |
| ET—1963 | 29 | 128 | 16 | 16 | tr | 4,096 | 512 | 512 |
| | 1 yr | 12 | 0 | 0 | 0 | 128 | 64 | 16 |
| | 2 yr | 8 | 0 | 0 | 0 | 128 | 32 | 0 |
| 5. CR Typhus 8-23-70 | 4 | 0 | 64 | 0 | 0 | 0 | 0 | 0 |
| Vac: RMSF—28 yr, 42+ doses | | | (0) | (0) | (0) | (0) | (0) | (0) |
| ET—8 yr, 18+ doses (last, 1952) | 24 | 128 | 512 | 64 | 64 | 8,192 | 4,096 | 2,048 |
| | | | (0) | (0) | (0) | (1,024) | (0) | (512) |
| | 149 | 64 | 64 | 32 | 16 | 256 | 64 | 32 |
| | | | (0) | (0) | (0) | (0) | (0) | (0) |
| | 2 yr | 8 | 32 | 0 | 16 | 64 | 16 | 0 |
| | | | (0) | (0) | (0) | (0) | (0) | (0) |

[a] Vac, vaccination.

[b] CF antibody titers to *R. rickettsii*, R strain (RMSF patients) or *R. prowazekii*, Breinl strain (typhus patients). 0 = titers < 1:6.

[c] Numbers not in parentheses indicate titers of antibodies undifferentiated as to Ig, and IgM class titers are indicated in parentheses. IgM antibodies were detected only in patient no. 5.

vidual organisms is visualized. The micro-IF test permits direct comparison of such interaction with different populations of organisms.

Both qualitative and quantitative characteristics of antigen-antibody interaction indicate the specificity of the IF reaction. Rickettsiae are complex organisms and probably possess many surface antigens differing among strains in kind and number. The extent to which antigens are shared determines the degree of relatedness. Theoretically, specificity of antigen-antibody reactions is determined by the degree to which the host immunologically responds to various antigenic components of the infecting strain, availability of the kinds of antibody needed to combine with all surface antigens of the test strain, and avidity of antibody for specific antigenic determinants. If these assumptions are correct, then rickettsiae in test antigens that stain sharply, uniformly, and intensely with convalescent serum are more likely to resemble infecting strains than rickettsiae with poorer staining characteristics.

Convalescent-phase human sera in this study generally showed rather broad reactivity to all strains within either the typhus or spotted fever group. Goldwasser and Shepard (7) noted difficulty in differentiating epidemic from murine typhus infections by IF. In their studies, the shared antibody was removed by absorption with heterologous strains. In our study, the sera from Ethiopian ET cases cross-reacted extensively with both *R. typhi* and *R. canada*. However, in every instance titers were as high or higher against *R. prowazekii*, and staining characteristics appeared to be more specific

against this rickettsia than against *R. typhi* and *R. canada*. We have not had an opportunity to test sera from murine typhus patients that were known not to have received ET vaccine to determine whether they would react more specifically against *R. typhi* than against *R. prowazekii*.

Although cross-reactions among spotted fever group antigens were usually quite extensive, sera from some convalescent RMSF patients reacted rather specifically to *R. rickettsii*, and antibody titers in all instances were at least as high against this organism as they were against other spotted fever group rickettsiae. The fact that three isolates from RMSF patients were indistinguishable in reaction patterns from the R strain of *R. rickettsii*, used as the standard antigen in all tests, is evidence that this strain is suitable for serodiagnosis of RMSF in the Rocky Mountain area. However, specific reactions to the R strain seemed to be less frequent in convalescent-phase sera from eastern patients. We have not yet had an opportunity to compare fresh isolates of western and eastern strains from RMSF patients to determine whether minor antigenic differences exist.

The remote relationship between typhus and spotted fever group rickettsiae, as evidenced by cross-reacting antibody responses to human illness, is well recognized. Cross-reactions were also observed in this study. In some instances, they seemed to be heterologous primary responses shown by presence of IgM antibodies. That others may have been secondary responses in persons who were previously sensitized by heterologous vaccination or infection was suggested by high Ig antibody titers without demonstrable IgM reactivity.

Variations in reaction patterns to heterologous strains within the spotted fever group were often noted. The reasons for this have not been established. Such differences in host response might reflect minor antigenic differences in infecting strains of rickettsiae, but this is not likely the only explanation. The three isolates from RMSF patients (Table 1) were antigenically indistinguishable, yet the breadth of antibody reactivity to other spotted fever and typhus group antigens in the corresponding patients varied.

Antibody response to illness in vaccinated persons is pertinent to this issue. Specificity seemed to be particularly pronounced in previously sensitized persons and was directed toward the antigen contained in the vaccine. Goldwasser and Shepard (7) observed that persons vaccinated against ET responded more vigorously to the vaccine strain than to *R. typhi* during subsequent infection with murine typhus rickettsiae. This phenomenon may partially explain why western patients with RMSF reacted more specifically to *R. rickettsii* than did most eastern patients. RMSF vaccine has been used quite extensively in the Rocky Mountain states, and information on previous vaccination was not obtained from most study participants.

Aside from antigenic conditioning, it seems plausible that individuals within a species as genetically complex as man will respond to antigenic stimuli in varied ways. There is no doubt that differences in response occur among host species. For example, the antibody response of mice to infection with certain spotted fever group and typhus group rickettsiae is more specific than that of humans (17; Philip et al., unpublished data). In cross tests of mouse antisera, *R. akari* and *R. canada* are completely distinct from other spotted fever and typhus group strains, whereas sera from RMSF and typhus patients frequently react with these organisms.

Murray et al. (13) showed that humans respond to primary typhus infection by early formation of 19S (IgM) antibodies. Tests with our anti-IgM conjugate indicated that most RMSF, as well as typhus, patients developed IgM antibodies. However, we could not detect an IgM response in about 15% of patients. Some Ethiopian cases may have been recrudescent typhus in which IgM antibodies were not recalled (13). We have no firm explanation for lack of IgM response in some RMSF cases. Steric hindrance of IgM reactivity by more avid IgG antibodies has been observed during convalescence from chlamydial infection (11). We did not fractionate sera into Ig classes to determine whether this sometimes occurs in rickettsial infection.

The duration of the IgM response was not clearly defined in this study. However, it was evident that the response varied. In one instance, IgM antibodies were demonstrated nearly 1 year after RMSF infection. Nevertheless, IgM antibodies are probably indicative of primary infection. Their determination should help to establish diagnosis of primary rickettsial infection, provided the conjugate is known to be specific and the patient has not been previously sensitized by vaccination or infection to related antigens.

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