

Human Serological Response to Louse-Borne Relapsing Fever

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Serological response to louse-borne relapsing fever in Ethiopia was determined by immobilization tests using *Borrelia recurrentis* cultures. Isolates from 26 patients tested with autologous convalescent sera showed from 90 to 100% of the organisms had been immobilized. Sera from thirteen patients were tested with autologous and heterologous strains. Several reacted with the majority and two showed high titers against all strains tested. Screening of day 2 and day 8 sera frequently showed heterologous antibody present before autologous antibody appeared, suggesting multiple antigenic challenge to infected patients. Some strains appeared to be antigenically related, but because of the wide diversity of serological responses, no definite serological groupings could be ascertained.

Relapsing fevers are characterized by an initial period of fever and spirochtemia and an apparent spontaneous recovery, followed by one or more subsequent relapses in the untreated patient. The relapsing character of these diseases has been attributed to the antigenic instability of the invading *Borrelia* species (11); as the patient develops immunity to the primary infection, the strain adopts a new antigenic composition, "reinfects" the patient, and persists until antibody to the "new" antigen is produced. The concept of antigenic variation during the course of the disease was advanced by Russell (9), who found that recovery from both relapsing fever and trypanosome infections occurred after the development of high-titer lysins for several serological variants.

Melenev, in China, noted six antigenic variations in local louse-borne strains (8), Cunningham et al. found nine in India (5), and, using tick-borne strains, Coffey and Eveland reported four types emerging in a definite sequence when rats were infected with one antigenic variant of an American strain (4). Schuhardt and Wilkerson observed several types after infection with only one organism (10); others have reported that antigenic change in relapse strains does not always occur (1).

The present study was conducted in Addis Ababa, Ethiopia, where louse-borne relapsing fever has remained endemic in the highland population since 1937 (13). By the development of a bacterial culture medium (6), it was possible to maintain strains for a limited time and to obtain sufficient antigen for serological studies. We attempted to determine, by immobilization tests similar to those of Vaisman and Hamelin (14) and of Felsenfeld (7), whether a definite strain-specific response could be detected in humans infected with *Borrelia recurrentis* and whether serological types could be characterized by this response.

MATERIALS AND METHODS

Patients. The study population consisted of persons with louse-borne relapsing fever admitted to the U.S. Naval Medical Research Unit no. 3 Research Detachment's Clinical Unit at St. Paul's Hospital in Addis Ababa. During the period from January 1972 to June 1973, approximately 100 patients were under observation. The serological studies reported here were performed on consecutive patients admitted from February to April 1973, and represents all cases during this period in which we were able to obtain both cultures and convalescent sera, with the exception of one (no. 30) who had been admitted in the previous year. Patients had a mean duration of symptoms of 5.4 days before seeking medical aid and a spirochetemia ranging from 5,000 to 140,000/mm³ upon admission. None of these patients gave a history of previous infection, and none were in the relapse phase of the disease. After blood was drawn for initial studies, including culture for *B. recurrentis* and other pathogens, a single dose of erythromycin was given and supportive management was initiated. Serum was drawn on days 2 and 8 after treatment.

Antigen preparation and serological tests. Venous blood was withdrawn into vacutainer tubes containing sodium polyanethol sulfonate (Becton, Dickinson & Co., Rutherford, N.J.). After centrifug-
ing at 900 rpm for 8 min, cultures were made by inoculating 1 ml of the supernatant plasma into 100-ml volumes of Trypticase soy broth containing 2% bovine albumin and 1% gelatin, as previously described (6). Incubation was at 36°C in an atmosphere of 0.4% CO₂. Portions of plasma were dispensed into sterile vials and maintained at -70°C in an equal volume of 8% glucose for culture at a later date.

Immobilizing tests were performed in triplicate. Sera from laboratory personnel were used as negative controls, and the autologous convalescent sera were used as the positive control. Cultures were used directly for immobilizing antigen after incubation from 2 to 4 days. Spirochete density was adjusted to contain 20 to 40 live organisms per field at a magnification of ×450 using a phase-contrast microscope. The test was performed by adding equal amounts of serum and suspensions of live spirochetes (approximately 0.01 of each reagent) to depressions in concave slides. Capillary tubes, fitted with a suction bulb, were used for mixing the solutions by repeatedly withdrawing and expelling the contents. A measured amount of the mixture was deposited on a glass slide, fitted with a cover slip, and incubated in a moist chamber at 36°C for 30 min. Cultures of fluid medium without added serum were used as standard; the number of viable organisms in 8 to 10 fields was counted under the dark-field microscope at ×450, and twice as many fields were counted in the test specimen. Immobilization titer was calculated by the following equation: (number viable in standard minus number viable in test serum × 100)/(number viable in standard) = percentage immobilized.

RESULTS

Mixture of convalescent sera with bacteria from autologous cultures in the logarithmic phase of growth resulted in development of bulbular swellings and loss of motility, and the organisms appeared thin and lifeless. Sera were screened undiluted since dilution delayed death of *Borrelia*, organisms became irregularly clumped, and slides were difficult to read. Controls of normal serum occasionally gave titers up to 60% (Table 1). Twenty-six convalescent sera screened with autologous isolates showed immobilization from 90 to 100%.

Screening of convalescent sera with 13 heterologous strains was attempted to determine if antigenic types could be distinguished on the basis of response after human infection (Table 2). The ability of a serum to immobilize from 90 to 100% of the organisms was considered for the purposes of this study to represent an immunity to the strains tested. Patients 72 and 59 reacted with all of the strains. Patient 71 also showed multiple response but did not react with strain 70. Patients 69, 60, 63, 64, and 70 showed high titers to the majority of strains that patient 68 reacted with, yet each one in this group failed to react with at least one of the strains that 68 did. Patients 66, 65, and 58 showed high immobilization of corresponding isolates, indicating that these three patients probably had been infected with similar strains. The majority of patients

<table>
<thead>
<tr>
<th>Culture no.</th>
<th>Organisms immobilized (%)</th>
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<tbody>
<tr>
<td>72</td>
<td>100 95 100 60</td>
</tr>
<tr>
<td>59</td>
<td>100 100 75</td>
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<tr>
<td>71</td>
<td>100 100 100</td>
</tr>
<tr>
<td>68</td>
<td>100 100 85 100 100 100 70</td>
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<tr>
<td>69</td>
<td>100 100 100 95 100 80 100 70 100</td>
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<tr>
<td>60</td>
<td>100 100 75 100 80 100 60 60 70 60</td>
</tr>
<tr>
<td>63</td>
<td>90 100 100 100 100 100 60 100 100 50 80 70</td>
</tr>
<tr>
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<tr>
<td>66</td>
<td>90 95 85 90 95 90 50 95 95 100 100</td>
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<tr>
<td>65</td>
<td>100 100 100 100 100 100 80 100 80 80 70 90 90 100 100 50</td>
</tr>
<tr>
<td>58</td>
<td>100 90 100 100 100 100 80 100 100 60 95 95 95 95 95 95</td>
</tr>
<tr>
<td>30</td>
<td>90 100 60</td>
</tr>
</tbody>
</table>

* Serum number.
* Less than 50% of organisms immobilized.
reacted with these latter strains. Patient 30 gave a significant titer only to his own strain.

This series of isolates indicated a rough serological grouping: isolates 72, 59, and 71 comprising the first group, isolates 68, 69, 60, 63, 64, and 70 comprising the second group, 66, 65, and 58 comprising the third, and 30 being the only isolate from the fourth group.

The bilateral cross-reactions observed within these groups, in which two infected patients had high immobilizing antibody titers to each other's strains, could indicate that these strains were identical (Table 3). A one-sided cross-reaction was also frequently observed in this series. In this situation, serum from one person immobilized his own plus a second isolate, yet that from the person infected with the second isolate immobilized his isolate but not the first one. This suggests that the two strains do not possess identical antigenic determinants and the first person must have been exposed to a multiple antigenic challenge, possibly in the form of infection with multiple strains or antigenic change during the process of infection to the strain isolated in the laboratory.

Table 2 enumerates convalescent sera and cultures to which both bilateral and one-way crosses occurred. Patients 59 and 72 showed bilateral crosses to each other's strains, but their sera showed considerable immobilizing activity and were able to react with all cultures tested. Five other patients (71, 68, 69, 60, and 63) each reacted to at least four other strains, yet each individual patient showed bilateral relatedness to other strains less frequently. Patients 66, 65, and 58 reacted only with corresponding strains, suggesting identity or close serological relationship. Patient 30 showed neither bilateral crosses with another patient nor one-sided crosses with other cultures.

Sera, taken on the second day after treatment, were subsequently screened with reacting isolates to determine if heterologous immobilizing antibody appeared sequentially or concurrently. Figure 1 shows day 2 and day 8 titers of five patients. Three of these showed an initial reaction to strains other than the one isolated before the initiation of therapy. Patients 63 and 60 both reacted to three other strains, and a moderately high titer to their own isolate was present at that time. By the eighth day, an increase to one other strain was observed in patient 63 and to three others in patient 60. Patients 58, 64, and 65 showed response to two other strains appearing at day 8. These latter two patients showed concurrent increases and no significant antibody was demonstrated on day 2.

### DISCUSSION

Immunity to louse-borne relapsing fever has only rarely been studied. Sparrow (12) reported complete immunity against experimental infection in untreated patients, but those treated could be reinfected within 2 or 3 months. Balteau and co-workers (2) reported that convalescent human antisera administered to infected patients would result in effective cure. Two treated persons have been observed in our clinical ward to become reinfected (D. T. Dennis, personal communication), one after 2 months and the other after an interval of 2.5 years.

Several animal studies of experimental infection with tick-borne strains have demonstrated multiple response. Ashbel (1) noted that numerous relapses and strains isolated from successive relapses were capable of protecting against both relapse and original strains, although the original strain was unable to protect against the relapse strain. He suggests that relapse strains might contain original antigens of the first strain plus additional antigens. Chamsa (3) isolated three different strains from...
five ticks on one dog in a natural infection; two ticks carried a mixture of two of these strains. He suggested that strains might not be an "antigenic mosaic," but each strain might instead constitute a mixture or complex of different antigenic types. Coffey and Eveland (4), noting a reproducible sequential appearance of three additional types after injection of a known standard, postulated a genetic change as the mechanism, but could not eliminate the possibility of an original stable mixed population of serotypes.

No person under study in this series gave a history of previous infection and none were in the relapse phase. Many of our patients frequently demonstrating a plural antigenic response and the demonstration of the appearance of antibody to heterologous strains prior to the appearance of antibody to autologous strains suggests either previous subclinical infection or antigenic change in the infecting strains during the prolonged infection without the intervention of relapse. The concurrent appearance of antibody to multiple strains could suggest that some strains are closely related antigenically, but a definite serological classification could not be made.

Another possibility for multiple response could be that some bacterial isolates may have constituted a mixed population of antigenic types coexisting during infection. The presence of serum-fast variants, although not generally observed, has been noted by Cunningham et al. with louse-borne strains (5) and by Schuhardt and Wilkerson with tick-borne strains (10).

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LITERATURE CITED


