The serological response to *Yersinia pestis* infection*

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Passive haemagglutination antibody titres to Fraction I antigen of *Yersinia pestis* were plotted against day of clinical illness in 82 patients in Viet Nam. A rise was evident by day 5 with a peak at day 14, after which a plateau occurred. In contrast to all other patients, 2 patients with recurrent infections had elevated titres at the time of admission which decreased significantly during convalescence.

Plague infection is distributed worldwide and is characterized by the abrupt onset of fever and regional lymphadenitis. The Fraction I antigen of *Yersinia pestis* is a protein with antiphagocytic properties located in the capsular envelope of the bacterium (1, 2). Antibody titres against this antigen are widely used for the serological diagnosis of plague and as a measure of immunity following vaccination (3, 4). In this study, sera were obtained from patients with suspected bubonic plague at the time of admission to hospital and prior to discharge. The kinetics of the passive haemagglutination antibody response against Fraction I antigen was described and the diagnostic usefulness of serology examined.

**MATERIALS AND METHODS**

**Patients**

All patients studied were Vietnamese patients admitted to the Danang Medical Center, Danang, Viet Nam or to the Cho-Quan Hospital, Saigon, Viet Nam, between 1970 and 1975. All were suspected of having plague because of fever and bubo. The diagnosis was confirmed by a positive culture of a bubo aspirate or blood for *Y. pestis*, identified by the bacteriological methods described by Sonnenwirth (5). Patients with a negative culture were confirmed by determining the passive haemagglutination (PHA) antibody titre against Fraction I of *Y. pestis*; those that showed a 4-fold or greater increase between the time of admission and convalescence or were \( > 1:16 \) on any single determination were considered positive. All patients denied receiving immunization against plague.

**Serological responses**

PHA antibody titres were measured using *Y. pestis* antiserum as a positive control and *Y. pseudotuberculosis* and *Y. enterocolitica* sera as negative controls (6). All positive sera showed complete inhibition by antigen inhibition tests. The means of the logarithms (base 2) were calculated for each day of illness on which the sera were collected. A plot of mean titres against days of illness was constructed and a Gompertz curve was fitted by a method of least squares (7) computed with a Hewlett-Packard HP-65 programmable calculator.

**RESULTS**

**Serological responses**

Serum was obtained at the time of hospital admission from 82 patients, who were subsequently shown to have *Y. pestis* infection. Cultures of bubo aspirate were positive in 70 cases, blood cultures were positive in 2 cases, a cerebrospinal fluid culture was positive in 1, and the PHA antibody titre against Fraction I of *Y. pestis* increased 4-fold or more during hospitalization in 5 cases and was elevated in a single determination to \( > 1:16 \) in 4 cases. Two patients died. A second serum specimen (convalescent serum) was obtained at the time of discharge from 47 patients. The day of illness, as determined by the duration of fever or bubo, on which the admission sera were obtained ranged from 1 to 10, with a mean of 3.5 days; the convalescent sera were taken on days 5 to 38 after the onset of illness, with a mean of 13.3 days. The means of the logarithms (base 2) of the PHA antibody titres for each day of illness were calculated and plotted against the day of
illness (Fig. 1). All points up to day 13 represent means of multiple determinations, but the points at days 14, 16, 17, and 19 are single determinations, and the values for days 21–30 and 31–40 were calculated from 4 and 3 determinations, respectively. The Gompertz best-fit curve shows that a rise in titre occurred as early as day 5 and increased progressively up to day 14. The steepest portion of the curve occurred between days 11 and 12. Although there were too few points after day 14 to continue the curve, the actual data suggest that a plateau occurs at this time and lasts up to day 40. The titre 1 : 16 (log$_2$ 16 = 4), which is considered to be minimum significant titre for the diagnosis of Y. pestis infection, was reached on day 8 of illness in these patients.

Recurrent infections

Two of the 82 patients studied gave a history of previous plague infection 4 and 6 years, respectively, before their present illness. In both cases the previous positive bubo cultures for Y. pestis were documented in the records of the Pasteur Institute in Saigon, and one patient had had a convalescent PHA antibody titre of 1 : 64. The PHA antibody titre of both these patients was significantly elevated at the time of admission (Table 1). These high titres at the time of admission contrast with those of the remaining 80 patients, who did not have a history of previous infection and who did not have a titre greater than 1 : 16 on day 1 of illness or greater than 1 : 32 on day 3 of illness. Both patients with a recurrent infection showed a significant decrease in PHA antibody titre (8-fold and 4-fold, respectively) when retested during convalescence. None of the other 45 patients, in whom paired sera were examined, showed a decrease in titre. In 40 of these patients, a 4-fold or greater increase in titre occurred between the time of admission and during convalescence, and in 5 patients no significant change occurred. Both patients with recurrent infection were treated with streptomycin and recovered satisfactorily. Their illnesses were no less severe than those of other patients, and their response to therapy was no more rapid than that of the other patients.

DISCUSSION

The antibody responses assessed in this study were measured by the PHA test against the Fraction I antigen of Y. pestis. This antigen appears to have an important role in the pathogenesis of infection. It is in the capsular envelope, which is not present when the organism enters man from the flea but develops during the incubation period in the mononuclear cells of the lymph node (1). After development of the envelope, the Fraction I antigen allows the bacteria to resist polymorphonuclear phagocytosis. However, antibody against Fraction I is elaborated and neutralizes the antiphagocytic effect of Fraction I antigen. Thus, bacteria are rendered susceptible to death by phagocytosis.

Table 1. Recurrent Yersinia pestis infections

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<tr>
<th>Age (years)</th>
<th>Sex</th>
<th>Interval between infections (years)</th>
<th>Bubo culture</th>
<th>Log$_2$ PHA antibody titre to Fraction I of Y. pestis</th>
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<td>Admission</td>
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<td>Log$_2$ titre Day of illness</td>
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<td>4</td>
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<tr>
<td>70</td>
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This study demonstrated that antibody levels were too low to be detected at the onset of illness in most patients. As early as day 5 of illness, however, a rise in antibody was observed and continued steeply upward to reach a peak at day 14. Thereafter, there is evidence that a plateau occurred and persisted until at least day 40. The rapid rise in antibody titre in response to this infection demonstrates the diagnostic usefulness of obtaining paired sera in patients with an interval between sera as short as 7 days.

Protective immunity following recovery from *Y. pestis* infections has been assumed because of the very low incidence of reinfection reported in endemic areas. Furthermore, vaccines are protective, and the degree of protection against experimental infection has been correlated with antibody titres against Fraction I antigen (3, 4). Nevertheless, rare instances of recurrent plague have been reported (8). In our study we had the unusual opportunity of seeing 2 patients with previous documented infections. Both patients, in contrast to all others without previous infections, had an elevated titre at the time of admission and showed an unexpected decrease in titre during convalescence. The high titre at the time of admission is likely to represent an anamnestic rise due to immunological memory of their previous infections. The observed decline in titre during convalescence, however, is more puzzling and has not been previously noted in this infection. One of the patients had shown a normal increase in convalescent antibody titre when previously confined, thus eliminating the possibility of a specific deficiency in antibody response to *Y. pestis* fraction I antigen. It can be presumed that the previous titre had decreased to a negligible level in the years since primary infection had occurred. Two human plague cases in the USA, for instance, had convalescent titres of 1:128 and 1:512, respectively, that remained at these levels for 6 months to 1 year and gradually declined during the following 1–2 years to levels of 1:4 and 1:8, respectively (B. W. Hudson, unpublished data). Assuming an incubation period of 5 days before onset of symptoms and 1–3 days of illness before acute serum specimens were obtained, there would be ample time for an anamnestic antibody response to occur. Although the severity of illness in these two patients was no less than that in other patients, pre-existing immunity may have limited the replication and dissemination of the bacterial antigens, thus essentially limiting antigenic stimulation and resulting in an abortive antibody response.

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RÉSUMÉ

**RÉPONSE SÉROLOGIQUE A L’INFECTION PAR *YERSINIA PESTIS***

Bien qu’en matière de peste la culture soit la méthode diagnostique la plus sûre, une épreuve sérologique utilisant comme antigène la Fraction I de *Y. pestis* dans une réaction d’hémagglutination passive (HAP) peut servir tant au diagnostic qu’à la séro-épidémiologie. La présente étude avait pour objectif d’évaluer l’utilité de cette épreuve par l’examen de la cinétique de l’accroissement du titre des anticorps après le début de la maladie. Elle portait sur 82 malades vietnamiens présentant des signes de peste bubonique et hospitalisés dans les villes de Danang et Saïgon. Aucun d’entre d’eux n’avait été immunisé contre la peste.

Les moyennes des logarithmes (base 2) des titres des anticorps HAP pour chaque jour de la maladie ont été calculées et portées en fonction du jour de la maladie. Une augmentation du titre apparaissait dès le jour 5 et s’accentuait progressivement jusqu’au jour 14, après quoi apparaissait un palier. Le titre de 1:16 (log_{16} = 4), qui est considéré comme le titre minimal significatif pour le diagnostic de peste, était atteint au jour 8 de la maladie.

Deux des 82 malades avaient des antécédents d’infections pestes (4 ans et 6 ans auparavant) confirmés par des dossiers signalant des cultures positives. A la différence...
rence de tous les autres malades, ces deux sujets présentaient des titres élevés au moment de l'admission et ces titres se sont abaissés considérablement au cours de la convalescence.

D'après ces constatations, l'accroissement rapide des titres HAP à l'égard de la Fraction I de Y. pestis permet de poser un diagnostic sérologique de peste précoce, à savoir une semaine après le début des symptômes. L'épreuve HAP peut également être utile pour étudier l'immunité dans des populations parmi lesquelles des infections récidivantes peuvent se produire.

REFERENCES