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The incidence and significance of Phase 1 complement-fixing antibody in Q fever

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INTRODUCTION

Early workers with Q fever (Robbins, Rustigian, Snyder & Smadel, 1946; Topping, Shepard & Huebner, 1946) reported puzzling differences in antigen sensitivity of various strains, so far as complement-fixing activity was concerned. The anomalies were largely explained by Stoker (1950, 1953), who showed that antigen prepared from early egg-passages of 'Christie' strain, whilst it combined readily with antibody, as assessed by agglutinability, could not fix complement with Christie antiserum unless the antibody was present in high concentration. After further egg-passage this ability to fix complement suddenly appeared. Stoker thought this might possibly be due to antigenic variation, with successive dominance of the two antigens, and considered that reported antigenic differences in strains may have been due to variations in the stage of egg adaptation. This view was reinforced when Stoker & Fiset (1956) demonstrated that the same phenomenon occurred with a 'Nine Mile' strain which had been continuously maintained by guinea-pig passage since isolation. These workers referred to organisms as being in Phase 1 when, in the early stages of egg adaptation, the rickettsiae reacted poorly with complement-fixing antibody, and in Phase 2 when, after egg adaptation, they reacted well. Stoker & Fiset found that organisms in Phase 2 could be reverted to Phase 1 by animal passage, and they believed that this was the naturally occurring state. This work was further elaborated by Fiset (1957) who showed that Phase 1 organisms contained both Phase 1 and 2 antigenic components. The former masked the latter, hence Phase 1 organisms would not, in early antisera, react with the phase 2 antibodies which they had elicited. During egg adaptation this surface component is apparently lost.

It has been known for some time (Robbins *et al.* 1946) that guinea-pigs developed C.F. antibody to Phase 1 antigen (as now realized) after a latent period of at least 40 days, but it was thought that this is a rare phenomenon in man. The demonstration by Andrews & Marmion (1959), and subsequently by Marmion, Higgins, Bridges & Edwards (1960), that patients with Q-fever endocarditis have C.F. antibody to Phase 1 antigen, has suggested that the presence of this antibody is indicative of chronic infection. It has seemed important to determine the frequency with which this antibody occurs, and to determine whether its presence can be correlated with any feature of the past illness or present clinical state.

MATERIAL

A follow-up survey was made of a consecutive series of seventy-two patients previously reported by one of us (Powell, 1960). It was possible to obtain sera for testing from fifty-one of these. Of the remainder, three had died of unrelated causes, nine could not be traced, and nine failed to report. Sera were tested for the presence of C.F. antibody to Phases 1 and 2 antigens.

TECHNIQUE

Complement-fixation tests were performed by the standard method of the Viral and Rickettsial Diseases Laboratory of the California State Department of Health (Jensen, 1956). Sera were inactivated at 60° C. for 30 min. and the tests incubated overnight at 4° C. Two exact units of fresh pooled guinea-pig complement in 0·2 ml., titrated in the presence of antigen, were used in the test. Phases 1 and 2 antigens prepared from the 'Nine Mile' strain of *Coxiella burneti* were supplied by the Commonwealth Serum Laboratories, Melbourne.

RESULTS

The results of C.F. tests with Phase 1 antigen on these fifty-one patients are set out in Table 1. It will be seen that 71% of the total did not have detectable amounts of antibody and only 10% had a titre beyond 1 in 8.

Of the fifty-one patients, the test was carried out at least 2 years from the onset of illness in all except four. Nine of the total, during the acute illness, had received treatment with one or another broad-spectrum antibiotics. To exclude any possible

Table 1. *Results of Phase* 1 C.F.T.

Phase 1 C.F. antibody titre	No. of patients
Negative	36
1 in 8	9
1 in 16	1
1 in 32	1
1 in 64	2
1 in 128	2
Total	51

Table 2. *Results of Phase* 1 C.F.T. *in untreated patients, arranged according to time after the acute illness*

Period since onset	No. of patients	Number with Phase 1 C.F. antibody*
22nd to 24th month	3	2 (8.16)
25th to 27th month	14	5 (8.8.8.64.128)
28th to 30th month	15	2 (8.64)
31st to 33rd month	6	3 (8.8.32)
34th to 36th month	4	1 (128)
Totals	42	13

* The figures in parentheses are the reciprocals of the individual titres.

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effects of treatment, the results are set out in Table 2 for the untreated cases alone, grouped according to the interval between onset and testing.

It will be seen that there is no significant difference in incidence or titre over the various 3-monthly periods.

Correlation with the original illness

No association could be detected between the following features of the acute illness and the subsequent development of Phase 1 antibody: duration of fever; erythrocyte sedimentation rate; abnormal liver function tests; the presence of hepatomegaly; the presence of splenomegaly.

Certain other features, however, showed an association, with varying degrees of significance.

Duration of convalescence

In the earlier study of this series it was demonstrated that the age of the patient influenced the duration of convalescence, which was longer in the older age-groups. In Table 3 is set out the period of incapacity for those patients for whom this information was available. The table is stratified according to age-group and according to the presence or absence of Phase 1 antibody.

Table 3. *Period of incapacity at the time of the original illness in those patients for whom this information is available*

	Period of incapacity (days)		
Age-group (years)	Phase 1 antibody absent	Phase 1 antibody present	Not tested
10–19	8.11.18.20.22.24. 27.30.30.41.42.44 (mean 26·4)	22.29 (mean 25·5)	30.32.39.40.58 (mean 39·8)
20–29	15.17.18.37.49 (mean 27·2)	18.18.44 (mean 26·7)	16.25.34 (mean 25·0)
30 and over	19.22.22.25.26.27. 28.29.32.32.34.40. 51.71.86 (mean 36·3)	20.31.99.114.119. 134.164 (mean 97·3)	19.20.28.29.40.40. 41.97 (mean 39·2)
Total patients	32	12	16
Mean period for all cases	31·2 days	67·7 days	36·75 days

There is a highly significant difference between the average length of incapacity in the age-group 30 years and over ($P < 0{\cdot}001$). There is no such difference in the younger age-groups. The fact that there is no significant difference between tested and untested patients in any age-group suggests that the tested patients are an unbiased sample of all patients in the series. It may be mentioned that the only patient in the 30–39 age-group with an extended period of incapacity (99 days) was aged 39 years.

Previous 'secondary' fever

Four of fifteen patients who subsequently developed Phase 1 antibody had experienced a secondary febrile period (Derrick, 1937) during the acute illness, as against four of thirty-six patients who were subsequently negative. Although suggestive, these figures do not reach significant proportions.

Severity of the original illness

Assessment of severity, except in terms of duration of fever, is subjective on the part of both patient and observer. Not all patients who appeared severely ill were subsequently found to have Phase 1 antibody, and, conversely, many of those who developed it had been only mildly ill. The over-all incidence of severe illness was much the same in the two groups. However, of the six patients with Phase 1 titres of 1:16 or greater, only two had had an illness and convalescence which was without incident. Two of the six had been jaundiced (the third jaundiced patient of the original series did not return for testing); a third, only mildly ill during the acute stage, had had a particularly long convalescence, and had been found to be excreting rickettsiae in the urine 3 months from onset; the fourth, at no stage particularly ill, had had evidence of rheumatic mitral valvular disease.

Correlation with present state

Only one patient with a titre of 1:16 or greater is known to be unwell, although one other of the six has not reported for interview, and a further one, who lives in another state, has been followed up by letter only. The man mentioned complains of persistent ill-health since the original illness, with frequent respiratory infections, malaise, and failure to gain weight; his present Phase 1 titre is 1:64. The man with mitral valvular disease has a titre of 1:128, but is quite well and shows no evidence of active endocarditis.

Table 4. *Results of simultaneous Phase* 1 *and Phase* 2 C.F. *antibody titres*

Phase 2 titre	No. of patients	No. with Phase 1 antibody*
Negative	15	Nil
1:8	7	Nil
1:16	12	3 (8.8.8)
1:32	13	8 (8.8.8.8.8.16.64.128)
1:64	4	4 (8.32.64.128)
Total	51	15

* Reciprocals of individual Phase 1 titres are shown in parentheses.

In a number of patients with detectable Phase 1 antibody attempts to isolate the organism by animal inoculation of blood and urine have all failed. This is possibly because Phase 1 antibody is a protective antibody, as shown by Abinanti & Marmion (1957).

There is a correlation between the Phase 1 C.F. antibody titre and that of a simultaneous Phase 2 C.F. antibody titre, as shown in Table 4.

All patients with positive Phase 1 C.F.T. also showed the presence of Phase 2 antibody, but the converse was not true. In general the Phase 2 titre was slightly higher than the corresponding Phase 1 titre, and in no case where the Phase 2 titre was negative or 1 in 8 was Phase 1 antibody detected.

DISCUSSION

It is clear that the finding of detectable amounts of Phase 1 complement-fixing antibody 2 years after infection is not a rare phenomenon. Moreover, it is obvious that such a finding is not of itself an indication of persistent overt infection and it is necessary to decide to what extent it is an indication of persistent latent infection. The association with protracted convalescence in the older patients may be of value in deciding this point. On the one hand, this could mean that in this age-group the more severe cases develop the antibody, and convalescence is prolonged because of the severity of the acute illness. This is unlikely, as the duration of convalescence is frequently quite out of proportion to the severity of the illness. Moreover, there is no correlation between the duration of fever and the subsequent development of antibody. On the other hand, the development of antibody may mean that infection has persisted beyond the period of the acute illness sufficiently long for this antibody response to have been initiated. On this view, the protracted convalescence in the elderly can be explained by the hypothesis that only in this group does persistent infection commonly produce manifest ill-health. The one case in whom rickettsiuria was found in convalescence would lend support to this view, as would the finding, in another series of cases (Powell, Kennedy, McIver & Silverstone, 1962), that the incidence of Phase 1 C.F. antibody 6 months or more after infection is significantly less in those patients who received treatment with tetracycline.

It should be realized that this hypothesis does not necessarily imply that infection is persisting at the time of testing, but only that it has persisted in the past. On general grounds, it can be suggested that the higher the Phase 1 antibody titre, the more likely is it that infection is still present, although latent. Such facts as the recovery of the organism from the placentae of parturient women (Syrucek, Sobeslavski & Gurvirth, 1948), and the occurrence of endocarditis in 1956 in a patient whose original illness was in 1945 (Powell, 1960), suggest that such latent infection may not be rare.

The finding of a high titre of Phase 1 antibody in a patient with mitral valvular disease but with no evidence of endocarditis is interesting, and we have also seen a similar phenomenon in another patient with mitral stenosis. A recent case (McIver, 1962) has shown us that active rickettsial infection of heart valves may be present for a considerable time without clinical evidence of active endocarditis. It has yet to be seen whether a similar process is occurring in these patients.

SUMMARY

1. An attempt has been made to follow up a consecutive series of seventy-two patients for the presence of Phase 1 C.F. antibody approximately 2 years or more after the acute illness. Fifty-one of the series were tested.

2. Fifteen of the fifty-one patients had detectable amounts of antibody, generally in low titre.

3. The presence of Phase 1 antibody correlated well, in older age-groups, with the duration of convalescence following the acute illness. There was no correlation with the duration of fever.

4. All cases with Phase 1 antibody also had Phase 2 antibody, usually in slightly higher titre. In no case in which Phase 2 antibody was absent or present in low titre was Phase 1 antibody found.

5. It is suggested that the presence of Phase 1 C.F. antibody is an indication of past persistent infection. It cannot necessarily be concluded that it is an indication of present persisting infection.

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