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ACTIVATION OF THE CLASSICAL AND ALTERNATE PATHWAYS OF COMPLEMENT BY *CORYNEBACTERIUM PARVUM*

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SUMMARY

The immunological adjuvant *Corynebacterium parvum* has been shown to activate the alternate pathway of complement in human and guinea-pig serum. Human serum in addition contains anti-*C. parvum* antibodies leading to activation of the classical complement pathway.

The possible role of a *C. parvum* derived polysaccharide in this activation is considered in relation to the biological effects of the micro-organism.

INTRODUCTION

A number of biological effects are associated with the administration of a killed vaccine of *Corynebacterium parvum* to experimental animals. These include adjuvant effect for both T cell- and B cell-dependent antigens (Howard, Scott & Christie, 1973) and the ability to induce 'anti-self' red cell activity in CBA mice (McCracken, McBride & Weir, 1971). There is also widespread proliferation of lymphoid cells, including macrophages, in response to *C. parvum* injection which results in marked hypertrophy of lymphoid tissues (Halpern *et al.*, 1964). Of particular interest is the ability of *C. parvum* to inhibit tumour growth in mice (Woodruff & Boak, 1966; Halpern *et al.*, 1966), and the effectiveness of this agent to influence dissemination of tumour in man is currently under investigation (*New Scientist*, 1973).

The mechanisms underlying these effects are still obscure as are the particular constituents of the micro-organism responsible. Our recent observation that a polysaccharide moiety extractable from a strain of *C. parvum* binds to tissue cells (McBride, Jones & Weir, 1974) suggests the need to consider the possible involvement of this material in the biological

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effects of the organism. Various polysaccharides such as inulin, zymosan and endotoxin are known to activate complement. In addition various polysaccharides have anti-tumour effects which are possibly a result of activation of complement components (Okuda *et al.*, 1972). In the present study we report a further biological activity of *C. parvum*, that is its capacity to activate directly the complement system in both human and guinea-pig serum.

MATERIALS AND METHODS

Assay of complement components

Individual components were assayed by the effective molecular titration method described by Rapp & Borsos (1970).

C3 proactivator was identified in immunoelectrophoresis using anti-C3 proactivator antibody (Hoechst Pharmaceuticals) (Götze & Muller-Eberhard, 1971).

Agglutination tests

Direct agglutination tests were performed in the presence of Difco latex 0·8 μm, 100 μl of latex suspension in 1 ml of suspension of *C. parvum* 3×10^{11} organisms/ml. The stock suspension is finally diluted 1/20 before use in the agglutination test (Woodruff, McBride & Dunbar, 1974).

C. parvum strain 10390 was obtained from the National Collection of type cultures (Colindale, England) and cultured as described previously (McBride *et al.*, 1974).

RESULTS

When increasing concentrations of a washed suspension of killed *C. parvum* 10390 were incubated with guinea-pig or human serum for 1 hr at 37°C and centrifuged, there was a dose-dependent decrease in the total haemolytic complement (CH_{50}) of the supernatant (Fig. 1). The levels of the individual complement (C) components C1, C4, C2 and C3 were then measured in pooled sera before and after treatment with a dose of 5×10^9 organisms per ml. Following treatment of human serum the level of C3 was greatly depleted as were the components of the 'classical pathway' C1, C4 and C2. In contrast, whereas guinea-pig C3 was also depleted after treatment there was apparent sparing of C1, C4 and C2 in this serum indicating activation via the 'alternate pathway' of complement (Table 1).

Evidence that human, but not guinea-pig serum, contained 'natural antibodies' to *C. parvum* was provided by direct agglutination tests. Agglutination of *C. parvum* was observed at a 1 in 32 dilution of the human serum pool whereas no agglutination was observed with the guinea-pig pool. Further evidence that antibodies to *C. parvum* were present in human serum and could account for 'classical pathway' activation was obtained by testing sera that had been repeatedly absorbed with the organisms in the presence of 0·04 M EDTA. The absorbed serum was dialysed against dextrose–gelatin veronal buffer containing Ca^{++} and Mg^{++} and divided into two. One half was treated with *C. parvum* at a concentration of 5×10^9 organisms per ml, while the other was left untreated. The C4 titres of the treated and the untreated control sera were similar, being 7750 and 6940 respectively. In contrast, the titres of the terminal components (C3–9) (Borsos & Rapp, 1967) were 41 and 194 respectively indicating activation of the 'alternate pathway' of complement. These experiments therefore suggest 'that *C. parvum* has the capacity, in human serum, of activating

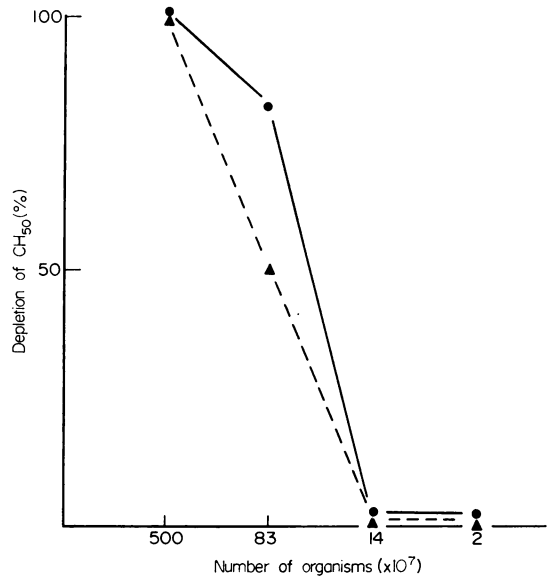

FIG. 1. Depletion of the CH_{50} of human (▲) and guinea-pig (●) serum by *C. parvum*. The CH_{50} was measured by the method of Mayer (1961). Each estimation was performed on a pool prepared from equal volumes of fifty human and twenty guinea-pig sera.

both pathways of complement'. These observations were further substantiated by incubating the human serum with the organisms in the presence of Mg^{++} EDTA, which permits 'alternate' (but not the Ca^{++}-dependent 'classical') pathway activation. The C3–9 titre of the serum that had been treated with *C. parvum* in the presence of Mg^{++} EDTA was <5 compared with the untreated control titre of 115, again indicating terminal component activation. Following immunoelectrophoresis using an antibody against the C3 proactivator (Hoechst Pharmaceuticals) the electrophoretic mobility of this protein had changed in the serum treated with *C. parvum* from the β to the γ region; an observation compatible with 'alternate pathway' activation (Götze & Muller-Eberhard, 1971). Thus the difference in the pathways of complement activation between the sera of man and guinea-pig is most likely due to the presence of 'natural antibodies' to *C. parvum* present in the human but not in the guinea-pig serum.

TABLE 1. The effect of *C. parvum* on the titre of the individual complement components C1, C4, C2 and C3 in human and guinea-pig serum

	Human serum		Guinea-pig serum	
	C. parvum treated	Control	*C. parvum* treated	Control
C1	11700	208000	22261	19321
C4	<5000	17021	9142	10240
C2	<50	1143	30567	35310
C3	305	2250	<50	1650

DISCUSSION

These results show that the immunological adjuvant *C. parvum* is capable of activating the alternate pathway of complement in both human and guinea-pig serum. The activation of the classical pathway in human serum is likely to be due to anti-*C. parvum* antibodies present in such sera.

It has been suggested that receptors for C3 on lymphoid cells may play a role in the antigen induction phase of the immune response (Pepys, 1972; Dukor *et al.*, 1974). A *C. parvum*-derived polysaccharide, bound to these cells (McBride *et al.*, 1974), and activating C3 may result in an amplification of the activity of these cells and also account for some of the biological effects of this agent. Whether or not the observed anti-tumour effect referred to above depends upon the presence of an intact complement system has yet to be determined. This study raises a number of issues relating to the biological activities of corynebacteria, some of which may be concerned in the initiation and amplification of cellular defence mechanisms. In addition this report points to a possible hazard in the use of *C. parvum* as a therapeutic agent, due to the formation of immune complexes with subsequent activation of the complement system.

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