The Role of Antibodies and Serum Complement in the Interaction between Macrophages and Leptospires

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Guinea-pig macrophages exerted bactericidal activity against both a virulent and a saprophytic strain of leptospira in the presence of the homologous IgG. Serum complement alone rendered the saprophytic strain susceptible to phagocytosis by the same macrophages.

INTRODUCTION

Recent studies on the interaction between guinea-pig macrophages and leptospires have shown that the phagocytic cells had no bactericidal effect on either the virulent strain PB-3 (serovar copenhageni of Leptospira interrogans) or the saprophytic strain Isola Sacra 1 (serovar isolasacra of Leptospira biflexa). The same macrophages did, however, exhibit a weak phagocytic activity against these two strains (Cinco et al., 1981). The present paper records the influence of serum complement and homologous IgG on the bactericidal and phagocytic activity of guinea-pig macrophages.

METHODS

Organisms and media. The characterization of the virulent leptospira strain PB-3 and of the saprophytic strain Isola Sacra 1, both of our reference collection, together with the method of culture and preparation of bacterial suspensions therefrom were described by Cinco et al. (1981).

Macrophages. Suspensions of guinea-pig macrophages were prepared and tested for their phagocytic ability before use, as previously described (Cinco et al., 1981).

Preparation of specific IgM and IgG. Three intravenous injections of about 107 leptospires, contained in 1 ml, of each strain, previously washed and resuspended in phosphate buffered saline pH 7-0 (PBS), were given to rats at intervals of 5 d. The animals were bled on day 40. The IgM and IgG fractions were separated from each immune serum on a Sephadex G-200 column (60 × 1-8 cm) equilibrated with 0-1 M-Tris/HCl and 0-2 M-NaCl, and characterized by immunoelectrophoresis.

The activity of each immunoglobulin preparation was tested against the homologous organism by the microscopic agglutination method of Petelin et al. (1970).

Complement. The source of this was pooled fresh guinea-pig serum absorbed twice with 5% (v/v) of a suspension of 1010 leptospires ml⁻¹ at 4°C for 15 min. The serum was then freed of leptospires by centrifugation and stored at −70°C till required. Complement activity was titrated by the Mayer haemolytic assay system (Lachmann & Hobart, 1978).

Determination of the opsonizing ability of IgG and of complement. Suspensions of both strains of leptospires at a concentration of 107 organisms ml⁻¹ in Krebs-Ringer phosphate solution (KRP) pH 7-4 were incubated with sub-agglutinating doses of homologous IgG for 30 min at 37°C. The leptospires were then harvested, washed and resuspended in KRP to a concentration of 1 × 10⁷ to 3 × 10⁷ leptospires ml⁻¹, to be used in the assay of the bactericidal and phagocytic activity of macrophages. In similar experiments with complement there was no period of pretreatment of the leptospires. The same amount of a similar concentration of untreated leptospires in KRP replaced the IgG-treated organisms in the leptospires/macrophages mixture, to which 10% (v/v) complement was then added. A 10% (v/v) concentration of complement was chosen since preliminary experiments had shown that
complement alone when diluted no more than six fold had a bactericidal effect on the saprophytic leptospira strain Isola Sacra 1.

Assessment of bactericidal and phagocytic activity of macrophages. As previously described, these two activities of macrophages were studied using as test system a mixture of equal volumes of a suspension in KRP of $1 \times 10^7$ to $3 \times 10^7$ leptospires ml$^{-1}$ and of a suspension in KRP of $2 \times 10^6$ to $4 \times 10^6$ macrophages ml$^{-1}$, with incubation at $37$ °C. At specified time intervals samples were taken and centrifuged, and the numbers of viable leptospires in the pellet of macrophages and in the supernatant were determined by culture on medium PLMS, thus giving an estimate of the phagocytic and bactericidal activity of the macrophages (Cinco et al., 1981).

Statistical analysis. The results obtained were analysed as previously (Cinco et al., 1981).

RESULTS

Influence of complement on the bactericidal and phagocytic activity of macrophages. Incubation of virulent leptospires with macrophages and complement at $37$ °C for $120$ min had no effect on their viability, whether they were free or in association with the macrophages. By contrast, after a similar period of incubation, there was a significant reduction ($P = 0.001$) in the number of colony-forming units (c.f.u.) of saprophytic leptospires ml$^{-1}$, both associated with the macrophages and in the liquid phase (supernatant) of the mixture of leptospires and macrophages (Fig. 1).

Influence of homologous leptospiral IgG on the bactericidal and phagocytic activity of macrophages. Following pretreatment with homologous IgG, the incubation of both the virulent and the saprophytic strain of leptospira with a suspension of macrophages at $37$ °C led, in the space of $60$ min, to a significant reduction ($P = 0.001$) in the number of c.f.u. ml$^{-1}$ in the mixture of leptospires and macrophages (Fig. 2). When pretreated leptospires alone were incubated at $37$ °C for $60$ min, there was no reduction in the number of c.f.u. ml$^{-1}$ in the suspension of organisms.

![Fig. 1. Phagocytosis and killing of leptospires incubated with a suspension of guinea-pig macrophages, in the presence of 10% (v/v) serum complement. After different periods of incubation, mixtures of leptospires and macrophages determined the number of leptospires in the pellet of macrophages and supernatant: virulent strain PB-3 pellet (■) and supernatant (○); saprophytic strain Isola Sacra 1, pellet (■), supernatant (□) and presence of serum complement alone (△). For all plots $P = 0.001$.](image1)

![Fig. 2. Phagocytosis and killing of leptospires after opsonization with IgG. Mixtures of leptospires and macrophages were treated as described in the legend to Fig. 1: virulent strain PB-3 pellet (■), and supernatant (○); saprophytic strain Isola Sacra 1 pellet (■) and supernatant (□). For all plots $P = 0.001$.](image2)
Fig. 3. Electron micrographs showing the phagocytosis of PB-3 leptospires opsonized with specific IgG by guinea-pig macrophages, after 60 min incubation at 37 °C. (a) Large phagocytic vacuoles (arrowed) containing numerous organisms; (b) phagocytic vacuole containing leptospires (arrowed) in different stages of degeneration. The bar marker represents 1 μm in (a) and 0.1 μm in (b).

Electron microscopy. Electron microscopy of macrophages to which leptospires pre-treated with homologous IgG had been exposed revealed that these cells possessed large phagocytic vacuoles containing numerous degenerate and lysed leptospires (Fig. 3).

DISCUSSION

The findings presented reveal that guinea-pig macrophages are able to ingest and kill both virulent and saprophytic leptospires when these organisms have been opsonized by...
homologous IgG. Serum complement alone, although capable of promoting not only the phagocytosis but also the killing of the saprophytic strain Isola Sacra 1, during the 120 min period of incubation, is unable to render the virulent strain PB-3 susceptible to these two activities of macrophages. Hence only specific IgG is able to opsonize virulent leptospires. Consequently the ability to escape phagocytosis could be considered a virulence factor for leptospires, responsible for their invasiveness in vivo, in addition to their resistance to the activity of complement (Johnson & Harris, 1967). It is therefore suggested that the antileptospiral antibodies synthesized during the early stage of the infection are responsible for the clearance of virulent leptospires from body fluids and tissues, when the illness passes from the septicaemic phase into the toxic phase. This stresses the importance of naturally acquired (Rottini et al., 1972) or artificially induced antibodies. Studies on the interaction between leptospires and human polymorphonuclear leucocytes, which may represent the first cellular defence mechanism against invading leptospires, are in progress.

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