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C-REACTIVE PROTEIN BINDS LEISHMANIAL EXCRETED FACTORS

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Abstract. Excreted factors from Leishmania tropica and Leishmania donovani are precipitated by human and rabbit C-reactive protein. The reaction is calcium dependent and appears to be similar to that reported to occur between C-reactive protein and various galactans. The absence of phosphate and N-acetyl galactosamine suggests that the reaction is not the result of any similarity of the excreted factors to pneumococcal C-polysaccharide.

Leishmanias are obligatory intracellular protozoan parasites that are responsible for visceral, cutaneous, and mucocutaneous diseases in man. Leishmania strains may be serotyped on the basis of a specific precipitin reaction between rabbit antisera raised against the whole parasites and a polyanionic, carbohydrate-rich substance, the excreted factor (EF) released into the culture medium of the organism (1-3). Galactose has been reported to be the immunodominant sugar of the EF of L. tropica and L. donovani (4). Our monosacharide analysis of EF samples revealed the presence of both galactose and xylose and suggested the possibility that these substances may be similar to galactans.

This possibility lead us to examine the interaction of the EF samples with C-reactive protein, a substance previously reported to interact with various galactans (5).

C-Reactive protein (CRP) was discovered more than 50 years ago by Tillet and Francis (6) and shown to form Ca^{++}-dependent insoluble complexes with a polysaccharide derived from the cell wall of Streptococcus pneumoniae (7). Plasma CRP levels increase rapidly in response to infections or tissue injury. CRP has been proposed to play an important role in the specific recognition of certain pathogens and necrotic cells of the host and in their subsequent elimination (8).

It has been suggested that EF on the surface of Leishmania promastigotes is involved in the attachment of the parasites to macrophages and in their subsequent ingestion (9). This report suggests a probable mechanism for such an effect, the binding of CRP to surface EF of the parasites.

Materials and Methods. EF was prepared by a modification of the method of Slutzky (3). After boiling and dialysis, the media were extracted three times at 68°C with 90% phenol. The aqueous phases were pooled, dialyzed against distilled water, and then chromatographed on a column of Sephadex G-50 equilibrated with 0.02M ammonium bicarbonate. The EF eluted in the void volume.

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CRP was purified from the ascitic fluid of patients with advanced metastatic carcinoma as described previously (10). The procedure involves affinity chromatography on a column of phosphorylcholine-Sepharose, ion-exchange chromatography on DEAE-cellulose and finally gel filtration on Sephadex G-200. Pneumococcal C-polysaccharide was isolated from rough Streptococcus pneumoniae, strain R36A (ATCC) by a slight modification of the method described by Liu and Gotschlich (11).

Double immunodiffusion studies were carried out in 1% agarose in 0.1M Tris-HCl, pH 8.0, containing 5mM $CaCl_2$. Approximately 20 µl samples were added to the wells and the plates were incubated overnight at room temperature. Capillary precipitation tests were carried out using 75 mm x 0.5 mm (ID) glass capillary tubes. CRP, EF samples, and pneumococcal C-polysaccharide were dissolved in 0.1M Tris-HCl buffer, pH 8.0, in the presence or absence of 5 mM $CaCl_2$. For some capillary precipitation experiments, the solutions also contained 4% polyethylene glycol 6000.

Phosphorus was analyzed by the Ames and Dubin procedure (12). Monosaccharide analysis of EF samples was carried out using a gas chromatographic procedure described previously (13).

Results. Fig. 1 illustrates the results of a double diffusion experiment in which the two EF samples and pneumococcal C-polysaccharide were allowed to react with CRP. Lines of complete identity were observed. This indicates that it was CRP, and not an impurity in the CRP preparation, that reacted with the EF polysaccharides. Precipitin bands were not obtained in the absence of calcium ions and the precipitin bands obtained with calcium ions present disappeared when the gels were soaked in buffer containing EDTA.

Since CRP is known to react with phosphorylcholine residues it was of interest to determine if phosphorylcholine was present in the EF samples. Chemical analysis of the EF samples, however, revealed no detectable phosphorus. In addition, the EF samples did not react with a monoclonal antibody directed against phosphorylcholine residues under the same conditions in which

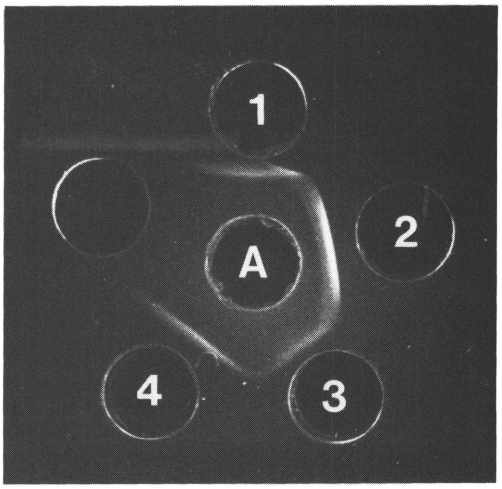

Fig. 1 Precipitin reactions of C-reactive protein (0.6 mg/ml) (A) with L. donovani E.F. (3 mg/ml) (1,4), pneumococcal C-polysaccharide (1 mg/ml) (2), and L. tropica E.F. (3 mg/ml) (3). Double diffusions were carried out in 1% agarose containing 0.1 M Tris-HCl, pH 8.0, and 5mM $CaCl_2$.

pneumococcal C-polysaccharide reacted strongly with the antibody. Monosaccharide analysis of the EF samples revealed the presence of galactose and xylose in an approximate molar ratio of 6:1 respectively, suggesting the presence of a galactan-like polysaccharide. No N-acetylgalacto-samine was detected in the EF samples.

Unlike human CRP, rabbit CRP does not react with most preparations of pneumococcal C-polysaccharide. Double diffusion experiments were therefore carried out in which the EF samples were diffused against both rabbit and human CRP. Rabbit CRP was found to react strongly with both Leishmanial excreted factors. However, unlike the human CRP it did not react with pneumococcal C polysaccharide.

A series of capillary precipitation experiments were carried out in which EF samples and CRP were tested against each other in various buffers. Unlike the strong reactions observed in the double diffusion experiments, only relatively weak reactions were observed in the capillary precipitation tests. However, the presence of 4% polyethylene glycol 6000 significantly enhanced the capillary

precipitation reactions. It is possible that the agarose gel itself also enhanced the precipitation of the two substances.

Discussion. Our results indicate that the excreted factors of L. tropica and L. donovani are bound by C-reactive protein and that the binding is calcium ion dependent. This finding may help explain several previous observations. For example, it has been suggested that the first line of defense after inoculation of L. donovani promastigotes into a nonimmune host is the non-specific binding of cross-reacting antibodies followed by activation of the classical pathway of complement (14). Several host defense mechanisms, including complement activation, are initiated following CRP binding to pneumococcal C-polysaccharide (8). It is possible that it is actually CRP that is responsible for this apparently non-specific effect of certain serum samples.

The fact that CRP binds to Leishmanial antigens may be of importance in host defense against these organisms but a more intriguing possibility is that this interaction actually benefits the parasite since macrophages generally take up particles coated with CRP (15). The reported interaction of surface EF on leishmania promastigotes with macrophages (9) may, in fact, be mediated by binding of CRP to surface EF of the parasites.

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