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Technical methods

Solid phase enzyme linked immunosorbent assay for classical complement activation pathway

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The complement system is a humoral defence mechanism found in vertebrates: it consists of nine proteinacous components termed C1 to C9, in addition to inhibiting and triggering factors.[1] Binding of the C1 component to immune complexes[2] or bacterial cell wall material[3] may activate the classical complement activation pathway. The early stages of the classical complement activation pathway are the activation C2 and C4 components by C1 and the subsequent activation of C3 by the activated $C4_2$ complex.[1] These activation steps precipitate a cascade of molecular events, resulting in complement mediated cell lysis and the liberation of anaphylotoxic substances.[1]

Assessment of serum complement concentrations is of diagnostic value in many autoimmune and hepatic disorders and in complement deficiencies.[1] Complement activity may simply be assayed by measuring the ability of sera to lyse antibody sensitised erythrocytes, which is then further characterised by a quantitation of specific components, usually C4 and C3, by immunodiffusion methods.[1] The assessment of the biological activity of individual components is, however, more difficult and requires complicated assays that may not be available in every laboratory.[1 4]

The classical complement activation pathway is initiated by the binding of the C1 subcomponent $C1_q$ to the Fc part of immunoglobulin,[1 2 5] whereas subsequent activation leads to the binding of C4 and C3 to the F(ab) part of immunoglobulin.[1 6] In this paper I describe an assay for functionally intact complement that measures the amount of $C1_q$, C4, and C3 bound to artificial γ-globulin aggregates.

Material and methods

SERA

Rabbit antisera, as well as fluorochrome conjugated rabbit immunoglobulins against human complement components $C1_q$, C4, and C3, were supplied by Behringwerke AG, West Germany. Alkaline phosphatase conjugated swine antirabbit immunoglobulin (the "conjugate") was supplied by Orion Diagnostica, Finland. Before use the conjugate was extensively absorbed with human γ-globulin (Kabi, Sweden) immobilised by cyanogen bromide coupling[7] to Sepharose 4B (Pharmacia Fine Chemicals, Sweden). Pools of normal human sera were used as the source of complement.

PREPARATION OF AGGREGATED γ-GLOBULIN

Human γ-globulin (Kabi, Sweden) was aggregated by incubating at 85°C for 30 minutes. The aggregates were further fractionated on a Sepharose G-200 column (Pharmacia Fine Chemicals, as previously described.[8]

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

The ELISA assay was performed on flat bottomed microtitre plates (Linbro, Flow Laboratories, USA) coated with aggregated γ-globulin in 0·01 M phosphate, 0·14 M sodium chloride buffer (pH 7·2) for three hours at 37°C. Coated plates were then incubated with pools of human sera diluted in phosphate buffered saline containing 0·05 % (v/v) Tween-20 (PBS-T) for two hours at 37°C. The plates were then incubated with antiserum against $C1_q$, C4, or C3 also diluted in PBS-T (two hours at 37°C), when the absorbed conjugate was added. After each treatment the plates were washed with PBS-T (three × 15 minutes). Conjugate bound to the solid phase was shown by the use of alkaline phosphatase substrate (p-nitrophenyl-phosphate, 1 mg/ml; Sigma Chemicals, USA).

IMMUNOFLUORESCENCE MICROSCOPY

Binding of complement components $C1_q$, C4, and C3 to capillary endothelium of human term placentae was assayed by immunofluorescence microscopy, as previously described,[4] and used as a comparative assay for functionally intact complement.

Results

STANDARDISATION OF THE ASSAY

A pool of human sera was tested in dilutions ranging from 1/10 to 1/1000 on microtitre plates treated in various ways to find the optimum dilutions of aggregated γ-globulin for coating anticomplement serum and conjugate. Plates were coated with aggregated γ-globulin at a concentration of 5 μg/ml, and rabbit anticomplement sera were used at a dilution of 1/300

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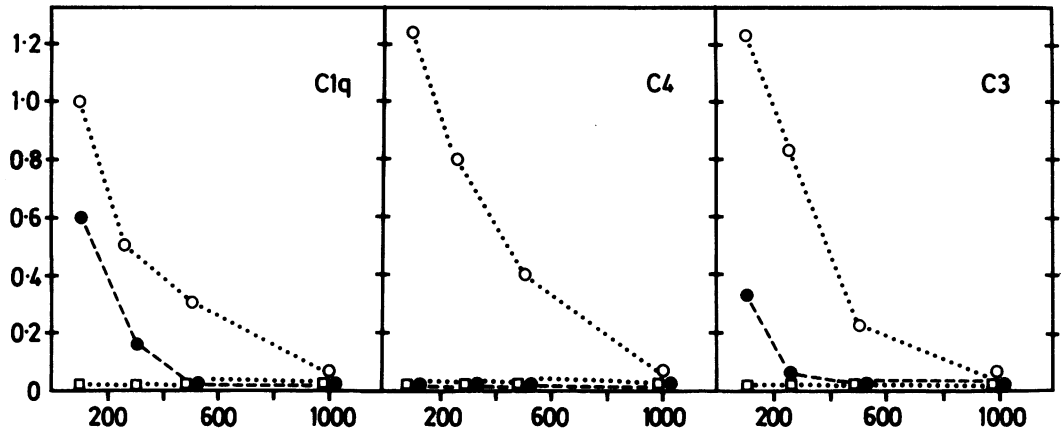

Fig 1 *Binding of complement components $C1_q$, C4 and C3 to solid phase aggregated γ-globulin. Vertical axes indicate absorbance at 405 nm; horizontal axes indicate reciprocity of serum dilution (complement source). Line symbols are as follows: ○ --- ○ serum diluted in PBS-T; ● --- ● serum diluted in PBS-T supplemented with 0·01 M edetic acid; □ --- □ heat inactivated serum diluted in PBS-T.*

and the conjugate at a dilution of 1/500, respectively. Under these conditions human serum pool dilutions ranging from 1/10 to 1/100 gave a uniform absorbance with anti-$C1_q$, C4, or C3, indicating that the complement binding epitopes on the solid phase γ-globulins were saturated. Dilutions ranging from 1/100 to 1/500 showed a dose dependent decrease in the absorbance, whereas dilutions of 1/1000 usually gave absorbances close to the background (figs 1 and 2). In the comparative immunofluorescence assay serum pools were positive for all three components to a dilution of 1/80.

EFFECT OF INHIBITORS

Heat treatment of serum (56°C for 30 minutes) is known to destroy the C1 component of complement,[5] and, as expected, heat inactivated serum pools did not show any binding of the $C1_q$, C4, or C3 components (fig 1). Celation of divalent cations also interferes with complement activation as the C1 component is disintegrated into its subcomponents in the absence of Ca^{++}.[1] In the presence of 10 mM edetic acid the binding of C4 was abolished, whereas the binding of $C1_q$ was weaker, and that of C3 clearly decreased (fig 1).

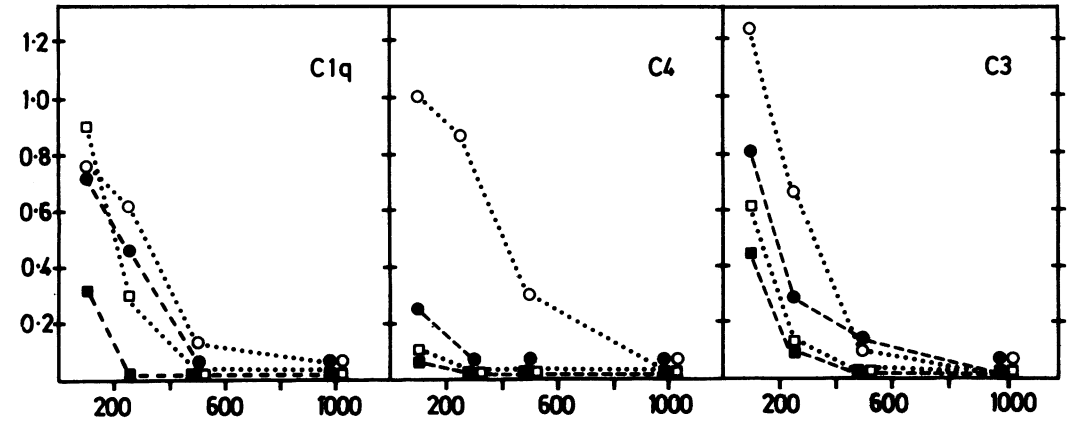

Fig 2 *Effect of soluble aggregated γ-globulin on binding of complement to solid phase aggregated γ-globulin. Axes are the same as those in fig 1. Line symbols are: ○ --- ○ serum diluted in PBS-T; ● --- ● serum diluted in phosphate buffered saline containing 1 μg/ml; □ --- □ = 2·5 μg/ml; and ■ --- ■ = 5 μg/ml of aggregated γ-globulin. Samples were preincubated at +37°C for 30 minutes before testing.*

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Discussion

The weakening of the $C1_q$ binding may be explained by the increase in ionic strength (which is known to decrease $C1_q$ binding,[2] caused by the addition of edetic acid and subsequent neutralisation of the buffer. The relatively high concentration of C3 in serum and the cascade nature of the classical complement activation pathway may explain the C3-binding observed in this experiment. In any event, the binding of complement components to aggregated γ-globulin did not seem to be due to non-specific adherance, but rather the biological integrity of the classical complement activation pathway.

The ability of the assay to measure different rates of complement consumption was measured by preincubating the complement source (37°C for 30 minutes) in the presence of aggregated γ-globulin at concentrations of 1, 2·5, and 10 μg/ml. In these experiments a clear dose dependent decrease in complement activity was observed (fig 2). Much higher concentrations of aggregated γ-globulin were needed to cause a decrease in complement binding in the immunofluorescence assay (100–500 μg/ml for $C1_q$ and 100 μg/ml for C4 and C3.[4] The different inhibition profiles may reflect differences in the complement binding structures used in the assays.

The ELISA method described here only responded to functionally intact complement and at much lower serum concentrations than the immunofluorescence assay (fig 1). With minor modifications—that is, by changing the anticomplement serum—the same assay records three different components of the classical complement activation pathway ($C1_q$, C4, and C3). The assay also recorded different amounts of complement activity (fig 2), indicating that it could become a simple and useful method for the measurement of complement activity in serum samples.

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Bacterial identification using nitrocellulose blotting technique that incorporates ELISA to bacterial surface antigen

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A major and recurring problem in microbiological diagnosis is the detection of a particular microorganism amongst a vast excess of irrelevant organisms. This is usually overcome by the use of selective media or judicious choice of culture conditions, or both. Several immunological techniques have also been used, particularly in the field of recombinant DNA technology.[1–4] In this context it is necessary to identify a small number of colonies making the required gene product from a library of several hundred thousand bacterial colonies. In one widely used technique a nitrocellulose sheet is applied to the surface of the bacterial culture plate and then peeled off, leaving most of the bacteria behind but transferring a small proportion of every bacterial colony on to the nitrocellulose replica. The bacteria on this replica are then lysed to release the recombinant protein, which then binds to the nitrocellulose sheet. By using antibody to the recombinant protein followed by enzyme labelled or radiolabelled second antibody, colonies making the desired protein can be identified. Viable bacteria can then be retrieved from the original agar plate.

Although recombinant proteins are usually intracellular, the above methodology should be applicable to the immunodetection of bacterial surface antigens. When using the technique for this purpose, however,

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