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Complement-mediated adherence of immune complexes to human erythrocytes

Difference in the requirements for C4A and C4B

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The classical pathway of complement is required for the adherence of soluble tetanus toxoid (TT)-human anti-TT complexes to erythrocytes. Using human C4-deficient serum we compared the capacity of the two forms of human C4 (C4A and C4B) to mediate this function: C4A was shown to be 1.5-fold more efficient than C4B. In contrast, haemolysis by C4B was 3.7-fold more efficient than by C4A. Such large differences suggest that both forms are complementary, and that C4A is preferentially involved in the processing of immune complexes in humans.

Immune complex disease; C4

1. INTRODUCTION

At the time of complement activation, the C4 molecule will interact with at least 8 other proteins which determine C4 activation, covalent C4b binding, formation of the classical pathway C3 and C5 convertases and their inactivation [1]. Variations in the structure of C4 are likely to affect one or another of these steps, and differences in the haemolytic activity of various C4 alleles have been observed [2–5]. The two forms of human C4, C4A and C4B, have different reactivity with sheep erythrocytes and also with insoluble immune complexes (IC) [4,5]. Upon C4 activation, the intrachain thiol ester bond of nascent C4b becomes available for reaction with acceptor sites present on proteins and cell surfaces; C4B binds to acceptor hydroxyl groups more efficiently than C4A, which in contrast reacts better with amino groups; these findings explain that C4B is several fold more efficient in haemolytic assays (hydroxyl groups predominate on erythrocytes) [6,7]. C4A is, however, not a 'lesser C4' since it binds to insoluble IC and mediates inhibition of immune precipitation better than C4B [5]. Here we investigate the role of the 2 forms of C4 in their capacity to promote adherence of IC to erythrocytes.

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2. MATERIALS AND METHODS

The immune adherence assay was performed as follows: preformed soluble tetanus toxoid (TT)–human anti-TT complexes made in antibody excess (20 × equivalence) containing 64 ng TT were incubated at 37°C with 25 μl serum; aliquots were removed after various intervals of time and added to 100 μl of a 25% suspension of human erythrocytes in 10 mM sodium phosphate, 145 mM NaCl, pH 7.5 (PBS). After incubation for

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10 min at 4°C, 1 ml cold PBS was added, and the percentage binding of IC to erythrocytes was determined from the pellets and supernatants recovered after centrifugation at 400 × g for 3 min at 4°C. When measuring C4 function, the sera or proteins were added in percentage of the 25 μl of C4-deficient serum used in the assay.

The following sera were used: (i) human C2- and C4-deficient sera; (ii) serum of a patient with nephritic factor containing <5% C3; (iii) serum from a patient with paraproteinaemia and classical pathway depletion containing <25% C1 subcomponents and C2, undetectable haemolytic C4, but normal C3; (iv) normal and C4 deficient guinea-pig (GP) sera.

C4 was purified as described [8] and, when required, was inactivated with methylamine [9].

Haemolytic C4 was measured according to Gaither et al. [10], and antigenic C4 by single radial immunodiffusion using monospecific antiserum [5].

C4 phenotypes in different normal sera were defined as described by Awdeh and Alper [2].

3. RESULTS

In normal human serum (NHS) complement mediated the adherence of TT–anti-TT complexes to human erythrocytes (fig.1). This reaction was rapid, maximal in 5 min, and required C3 and classical pathway function: no adherence was observed in the Nef-containing serum, C2- and C4-deficient sera, and classical pathway-depleted serum. Using normal and C4-deficient GP sera provided similar results. In human and GP C4-deficient sera, the addition of small concentrations (1%) of purified human C4 were sufficient to normalize this function (fig.1). Inactivation of C4 with methylamine abolished the binding.

Adding 1% (0.25 μl) of NHS to C4-deficient sera nearly normalized the reaction as well – this amount of NHS produced no IC adherence on its own. That the limiting factor in 1% NHS was C4 was shown by re-establishing adherence with 1% of C2-deficient serum, Nef-containing serum, and normal GP serum, but not by GP C4-deficient serum and the human classical pathway-depleted serum.

Thus, the immune adherence assay could be used to titrate C4 function in various sera using in

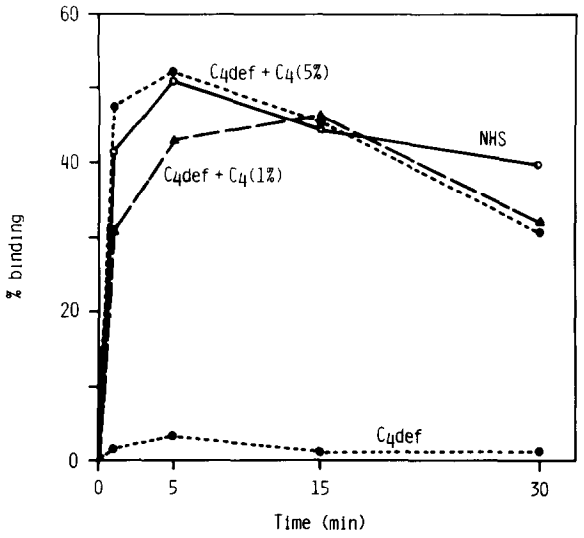

Fig.1. Binding of TT–anti-TT complexes to erythrocytes after various intervals of time in NHS, in human C4-deficient serum, and in this serum repleted with 1% or 5% purified C4.

the assay 25 μl human C4-deficient serum. C4 titration in NHS is shown in fig.2, and we arbitrarily defined 'functional C4-binding units' as the inverse of the concentration of serum required to produce 50% of the maximum binding (incuba-

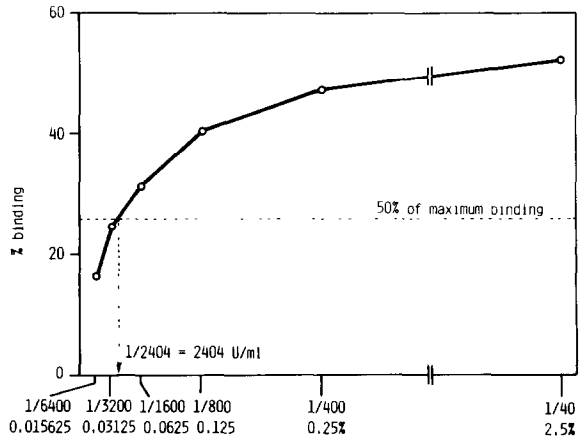

Fig.2. Adherence of TT–anti-TT complexes to erythrocytes after an incubation of 5 min with human C4-deficient serum supplemented with various dilutions of NHS. Functional C4-binding units in NHS were defined from the inverse of the dilution of the serum which restore 50% of the maximum adherence.

Table 1

C4 measurements

Sera	Phenotypes		Haemolytic activity (U/μg)	Binding activity (U/μg)	Ratio haemolytic/ binding activity
JS	A2A3	B1B2	90	6.3	14.3
C	A3	BQ0	42.1	7.0	6.0
D	A3	BQ0	50.3	8.6	5.9
S	AQ0	B1B2	169	5.2	32.5
A	AQ0	B1	203	5.1	39.8
N	AQ0	B1	144	5.0	28.8

tion time, 5 min). Normal human sera containing homozygous C4 null alleles were tested in this assay, and their functional C4-binding units were expressed per μg C4 (U/μg) (table 1): C4A was approx. 1.5-fold more efficient than C4B. In contrast, haemolysis by C4B was 3.7-fold more efficient than by C4A, so that the ratio of the two functions differed more than 5.5-fold for the two forms of C4.

4. DISCUSSION

Here we demonstrate that human C4A and C4B differ in their capacity to mediate immune adherence of soluble IC, and this observation is made in a system involving only human reagents – cells, antibodies, and complement source. The more efficient activity of C4A in this immune adherence assay corresponds to its more efficient binding to insoluble IC [3] and capacity to inhibit the precipitation of IC made with rabbit antibodies [5]. Thus, C4A is not a lesser form of C4, and is involved preferentially in the processing of IC.

In primates, the complement-mediated transport system for IC on erythrocytes has been shown to prevent IC deposition in various organs outside the fixed phagocytic system [11]. The immune adherence function of C4 described here is probably not limiting in the blood stream, since 1% C4 was sufficient; however, C4 could become limiting in extravascular spaces where complement protein concentrations are low, and where C3b binding to IC could be necessary for efficient recognition by phagocytic cells and those involved in the immune response; for instance, B cell memory has been shown to be defective in C3-depleted and C4- and C2-deficient animals [12,13]. It has been postulated that deficient C4 function is involved in the pathogenesis of autoimmune disease [1,14], a suggestion particularly entertained in patients with total C4 deficiency since most suffer from an SLE-like disease [15]. But are there any data on human diseases that suggest a different role for C4A and C4B? Four different studies indicate that homozygous C4A null alleles (but not homozygous C4B null alleles) are found more frequently in patients with SLE than in normal controls [16–19]. These observations would correlate with our in vitro findings: the absence of C4A – reacting preferentially with IC – may predispose to defective IC elimination.

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