ANTI-D-COATED Rh-POSITIVE RED CELLS WILL BIND THE FIRST COMPONENT OF THE COMPLEMENT PATHWAY, C1q

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1. Introduction

Human antibodies against the Rh blood group antigens do not in general activate the complement system. Rare reports of activation are restricted to either anti-D of certain sera bound to enzyme-treated red cells [1,2] or to anti-D bound to papain-treated red cell stroma [3]. Since C1q must bind through 2 of its 6 heads to 2 separate IgM molecules on the cell surface [4–6], it has been suggested that anti-D molecules are too far apart on the red cell surface to allow C1q to bind by two binding sites [7]. An alternative explanation is that C1 is able to bind to IgG anti-D on the cell surface but that there is failure in the subsequent activating steps. To differentiate between these 2 possibilities, the ability of ^{125}I-labelled C1q to bind anti-D coated red cells was investigated.

2. Materials and methods

A potent IgG anti-D preparation was further purified as in [8] followed by Sephadex G-200 column chromatography to remove aggregates. An aliquot was labelled with ^{131}I [9]. The concentration of the anti-D in the final preparation was 860 μg/ml. C1q was prepared and labelled with ^{125}I as in [10]. The specific activities of the anti-D and C1q were based on the use of absorbance values at 280 nm of 14 and 6.8 for 1 g/100 ml, respectively.

The red cells had the probable genotype R_1R_2 and *rr*. The saline used throughout contained 0.17 M NaCl, 0.003 M PO_4, bovine serum albumin 1 g/100 ml (pH 7.0), ionic strength 16 mM HO at 20°C. Red cell numbers were estimated using a Coulter electronic counter.

2.1. *A model of C1q binding to randomly distributed IgG molecules on the surface of red cells*

C1q must bind to 2 molecules on the cell surface. If the maximum span of the C1q molecules is r, then the number of C1q binding sites on the antibody-coated red cells will be given by the number of pairs of IgG molecules in which the 2 members of the pair lie within a distance r of each other. If the IgG anti-D molecules are randomly distributed on the red cell surface, then an expression giving the number of IgG molecules that lie as a pair within a distance r of each other can be derived from probability theory and is given by:

$$N - Ade^{-da}/2$$

where N = the total number of IgG molecules on the surface of each cell; A is the surface area of the red cell (taken as 163×10^{-12} m^2; [11]); $a = \pi r^2$ and d the density of the IgG/unit area of surface.

3. Results

3.1. *Binding of C1q to anti-D coated red cells*

In 4 separate experiments, red cells were heavily coated with unlabelled anti-D by adding an excess of antibody (~250–500 μg) for each 1 ml R_1R_2 Rh-positive cells, the latter having a total binding capacity of ~60 μg anti-D/ml cells [12]. After removing unbound anti-D by washing with saline, ^{125}I-labelled C1q was added and both the number of binding sites for C1q on the cells and the value of the functional equilibrium constant, K, was determined by Scatchard analysis as in [10]. Rh-negative cells were similarly treated and used as controls. The uptake of ^{125}I-labelled C1q

Table 1
Number of binding sites for ^{125}I-labelled C1q on Rh positive red cells heavily coated with non-radioactive anti-D and the value of K for the reaction

Expt	C1q binding sites/ red cell	Functional affinity constant K
1	1600	3.6×10^8 M^{-1}
2	1150	2×10^8 M^{-1}
3	720	4.2×10^8 M^{-1}
4	650	3.9×10^8 M^{-1}
		3.4×10^8 (av.)

by the Rh-negative cells was subtracted from the uptake on the Rh-positive cells. The estimate of the number of binding sites ranged from 650–1600 on each cell and the values of K varied from $2–4.2 \times 10^8$ M^{-1} (table 1). The variation in the number of binding sites resulted from the use of different anti-D to red cell ratios in each case.

3.2. *Relationship between C1q-binding sites and density of anti-D on cells*

To determine whether the number of available C1q-binding sites was a function of the amount of anti-D present on each cell, aliquots of cells were coated with varying amounts of ^{131}I-labelled anti-D and the number of antibody molecules/cell determined. The uptake of ^{125}I-labelled C1q by these cells at equilibrium was then determined and the number of available C1q-binding sites on the cells calculated using the law of mass action, since:

$$\text{Total C1q binding sites} = \frac{C1q_{bound} \times (1 + K \times C1q_{free})}{K \times C1q_{free}}$$

The pre-determined average value of 3.4×10^8 M^{-1} was used for K. Rh-negative cells were similarly treated to estimate non-specific uptake of radioactivity and these values subtracted from those obtained with the Rh-positive cells. The values obtained from this experiment as shown in table 2 and plotted in fig.1.

3.3. *Theoretical calculation of expected number of C1q-binding sites*

The expected number of C1q-binding sites with varying densities of anti-D molecules was calculated using the equation derived from probability theory.

Table 2
The relationship between the numbers of anti-D molecules/ cell and the number of C1q binding sites

Anti-D bound to red cells (molecules/cell)	C1q-binding sites cell
2.1×10^4	630
1.9×10^4	570
1.3×10^4	450
1.4×10^4	420
1.0×10^4	120
1.1×10^4	120

Calculations were made using values of r = 10,20,30 and 40 nm. The results of these calculations are shown in fig.1.

4. Discussion

These results demonstrate that C1q can bind to anti-D-coated red cells provided that the density of anti-D on the surface is sufficiently great. Owing to the non-specific uptake of C1q onto cells, the sensitivity of the method used here was such that the minimum number of C1q binding sites on each red

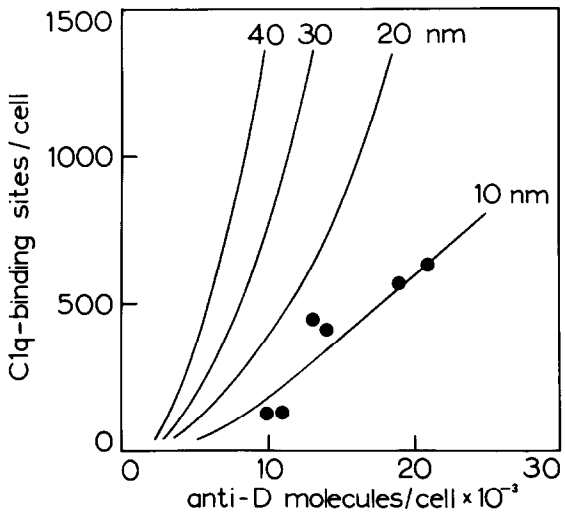

Fig.1. The relationship between the number of C1q-binding sites on Rh-positive cells and the number of anti-D molecules on each cell. The continuous lines are theoretical values (calculated using probability theory) giving the expected numbers of pairs in which the two members of the pair lie within 10,20,30 and 40 nm of each other; (•) observed values, using ^{125}I-labelled C1q and ^{131}I-labelled anti-D.

cell that could be detected with certainty was ~100. Approximately this number were detected when the number of anti-D molecules on each cell was ~10 000. At higher anti-D densities, the number of C1q binding sites increased rapidly so that with a doubling of the density to 20 000 anti-D molecules/cell, there was a 5–6-fold increase in C1q-binding sites.

The theoretical number of expected binding sites was calculated making two assumptions:

(i) That the anti-D is randomly distributed on the cell surface;

(ii) That the C1q must combine with 2 IgG molecules to form an adequately firm bond.

The dimensions given for C1q in [13] indicate that the molecule could span ~40 nm if fully stretched. A series of curves were therefore calculated to determine the number of pairs of IgG molecules that would lie within 10,20,30 and 40 nm of each other at various IgG densities. It can be seen from fig.1 that the observed binding of C1q fits the expected curve if the C1q molecules were only able to bind to pairs of IgG molecules lying within 10 nm of each other. It is to be expected however that C1q will not bind to all the available pairs of IgG molecules since it is likely that the combining regions on the C1q heads and on the IgG must be correctly orientated towards each other for a firm bond to take place. The orientation of the combining area on the 6 heads of C1q are probably fixed within narrow limits by their connections to the collagenous 'stalks' [13] and thus it is probable that once one of the heads has bound to an IgG molecule, the other IgG molecule must be correctly orientated to bind with a second head. The observed results are thus also compatible with the suggestion that C1q can combine with IgG molecules as far apart as 30 or 40 nm but that only some of the pairs are correctly orientated to allow binding to two heads.

The finding that C1q can combine with anti-D on the red cell surface is consistent with the findings [3] that papain-treated red cell stroma coated with anti-D will consume complement components. It is known that proteolytic enzymes bring about aggregation of the D-antigen on stroma [14] and this presumably results in many anti-D molecules bound to the antigen approaching sufficiently close to allow sufficient binding of C1 to activate the complement cascade.

The failure of anti-D to activate complement is thus not due to the inability to bind C1q but is probably due to a failure of activation of later stages. There may be a failure of C1r and C1s to activate even though C1 is bound (review [5]) or it may be due to inefficiency of C4 and C3 activation and deposition [15].

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