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The Possible Use of Complement for the Detection of Cell Surface Antigens[1,2]

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The modern trend in complement research concerns the functionally relevant interactions of complement components and the biologic activities of the complement system. The primary interest of students of complement today centers on complement as a humoral effector system which constitutes an integral part of the immunologically competent organism. There exists only a secondary interest at present in complement as a serologic reagent used in the laboratory for the detection of antigen-antibody complexes. Consequently, little work has been done so far on the use of complement for the detection of cellular antigens. The discussion of this subject will, therefore, have largely a theoretical character. The following presentation will include a brief description of the chemical nature of complement components and of the essential features of the complement reaction mechanism. On the basis of this information, those properties of individual complement components and of individual reaction steps will be pointed out which may be suitable to serve as sensitive indicators of antigen-antibody complexes and, hence, of cell surface antigens.

The complement system (most of the present discussion will deal with human complement) consists of eleven distinct serum proteins or nine complement components, which, in terms of protein, account for approximately 10% of the serum globulin fraction.[3] Of the eleven human complement proteins, seven have thus far been obtained in a high degree of molecular homogeneity [C'1q (25), C'1s (8), C'2 (17, 33, 35), C'3 (30), C'4 (23), C'5 (30) and C'9[4] (7)]. The other four components have been obtained in functionally pure form (18, 29, 31); however, they must await further chemical purification. Some of the properties of human complement proteins are listed in Table 1. None of these proteins appear to contain lipids; however, as far as investigated, they may contain a carbohydrate moiety. Specific antibody has been produced to C'1q (4, 21), C'1s (9), C'2 (19, 35), C'3 (24), C'4 (23), C'5 (30) and C'8 (18). Radioactive labels in the form of ^{131}I or ^{125}I have been introduced without appreciable impairment of the hemolytic activity into C'2 (35), C'3 (24), C'4 (24), C'5 (31) and C'9.[4]

When a cell is attacked by antibody and complement, the molecular events that precede lysis of the cell may be summarized as shown in Chart 1. An antibody molecule, A, attaches to a surface antigen, S, to form on the cell surface the antigen-antibody complex SA. The first component of complement, C'1, which consists of the subunits C'1q, C'1r and C'1s (15), then combines with the antibody portion of the cell-antibody complex. Upon interaction with antibody, C'1s, a proenzyme, is activated to C'1 esterase, or C'1sa (14). Activation of C'1s is most probably effected by an enzymatic mechanism, the responsible enzyme activity residing in C'1r (36). Next, C'1a, the activated first component, catalyzes the physical attachment to the cell surface of C'4 and then the physical attachment of C'2 to cell-bound C'4. Thus, there is the formation of the C'4,2a complex (27). The C'4,2a complex is endowed with enzyme activty, its natural substrate being C'3, and for this reason it is also called C'3 convertase. The enzyme catalyzes the physical attachment of C'3 to the cell surface, the formation of the C'4,2a,3 site, and the generation of C'3-dependent peptidase (5). In the next step, the latter enzyme apparently activates the fifth, sixth, and seventh components of complement, which tend to occur in physical association (31) and which, following "activation," act on the cell surface to render it susceptible to the action of C'8 and C'9. After C'8 and C'9 have become physically attached to the cell surface, these two components set in motion an intramembrane process of unknown nature which in turn leads to functional and structural breakdown of a localized membrane site[4] (6, 10). In the course of the entire reaction, several hemolytically inactive reaction products occur, only one of which is indicated in Chart 1: The cell-bound C'4,2a complex has a half-life at 37°C of approximately 10 minutes (20), and as it decays it dissociates a fragment of C'2 called C'2a^d (38), vacating the acceptor site on C'4 for a new molecule of C'2 (3). C'2a^d may be detected by competition with active C'2 for a neutralizing antibody to C'2 (19).

In order that complement can be utilized for the detection of cell surface antigens, the antibody used must meet certain requirements. Obviously, it must be capable of interacting with complement. Evidence has accumulated indicating that γA-type antibodies (11) do not react with complement and that

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[3] H. J. Müller-Eberhard, M. A. Calcott, and H. M. Grey, Interaction between C'1q and γG-Globulins of Different Heavy Chain Subtypes, manuscript in preparation.

[4] U. Hadding and H. J. Müller-Eberhard, The Ninth Component of Human Complement: Method of Isolation, Chemical Description and Mode of Action, manuscript in preparation.

Table 1

Properties	C'1q	C'1r	C'1s	C'2	C'3	C'4	C'5	C'6	C'7	C'8	C'9
Serum conc. (μg/ml)	100–200		22	10	1200	430	75				1–2
Sedimentation rate (S)	11.1	7	4	5.5	9.5	10	8.7	5–6	5–6	8	4.5
Approx. mol. wt.	400,000			115,000	240,000	230,000					79,000
Electrophoretic mobility	γ_2	β	α_2	β_2	β_1	β_1	β_1	β_2	β_2	γ_1	α
Carbohydrate conc. (%)	17				2.7	14	19				
Reactive SH				1 or more	2						

Properties of human complement components.

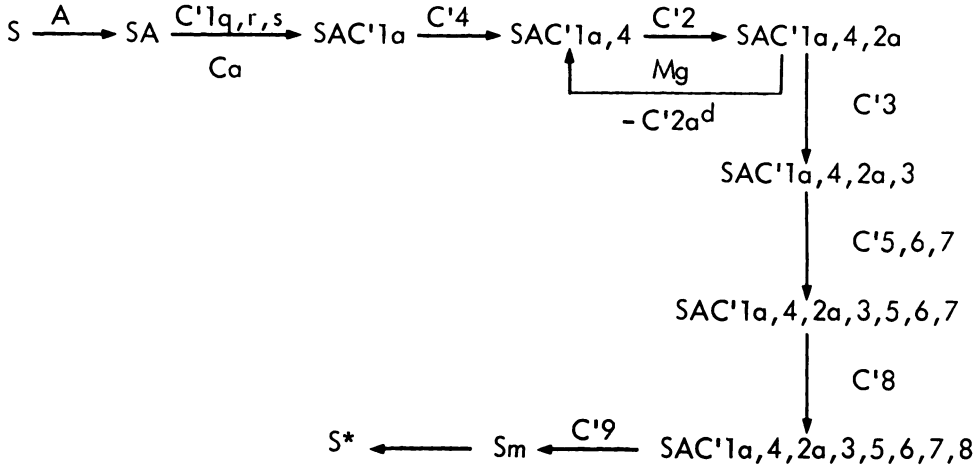

Chart 1. Membrane damage by complement.

for the reaction of γG-type antibodies with complement, at least two molecules of antibody must be placed in close proximity, while apparently only one molecule of γM-type antibody is needed to trigger the complement reaction (2, 10). Thus, if the antibody belongs to the γM class, its spatial arrangement on the cell surface may be irrelevant for the ensuing reaction with complement. In contrast, γG antibodies may be rendered nonreactive toward complement if the distances between the antigenic sites on the cell surface are too great to permit formation of fixed antibody doublets. In addition, it is known that γG-type antibodies vary in their reactivity with complement according to their heavy chain type[3] (2). This may be demonstrated by studying the reactivity of typed myeloma proteins with isolated C'1q.[3] While γG_1- and γG_3-globulins exhibited a pronounced affinity for C'1q, γG_4-globulins reacted poorly or not at all, and γG_2-globulin showed an intermediate reactivity. If an antibody to a cell surface antigen does not meet the requirements for complement fixation, these technical difficulties may be overcome by utilizing an antibody to the antibody. As a number of antibody molecules can be accommodated on one γ-globulin molecule, the conditions for complement fixation are usually fulfilled.

There are primarily two complement reaction steps that may be utilized as sensitive indicators for the presence of antigen-antibody complexes on the cell surface. The first reaction is the binding and activation of C'1, and the second is the formation on the cell surface of C'3 convertase. There are at least three methods for the detection of each of these two reactions.

Binding and activation of C'1 may be detected by C'1 transfer, as pointed out by Borsos and Rapp (1). Activated C'1 (C'1a) tends to dissociate from its complexes with sensitized cells, the equilibrium between cell-bound C'1a and free C'1a depending on the ionic strength of the buffer. While at physiologic ionic strength a considerable proportion of C'1a is unbound, most of the C'1a molecules become cell-bound as the ionic strength is reduced to half the physiologic value. To detect an antigen-antibody complex on the surface of a cell, complement is first added at low ionic strength to favor binding and activation of C'1. The cells are washed at low ionic strength to remove all unbound C'1 and are then mixed, at physiologic ionic strength, with sensitized sheep erythrocytes containing the fourth component of complement to allow transfer of C'1a to these indicator cells. Assay of C'1a on the surface of sensitized sheep erythrocytes is then carried out according to standard procedures. It is said that one molecule of C'1a per cell is sufficient to cause lysis of 63% of the cells present under optimal conditions.

Since C'1a is an enzyme and since C'2 and C'4 are its two natural substrates, it is obvious that activation of C'1 may also be detected by its effect on the two substrates. If purified C'2 or a serum fraction containing C'2 is incubated with C'1a, C'2 is rapidly inactivated (16, 37) by cleavage of the molecule into at least two fragments (35, 38). The degree of reduction

of C'2 activity during the period of incubation may be measured by standard procedures using sensitized sheep erythrocytes containing on their surface the first and fourth component of complement. Similarly, activation of C'1a may be measured by its effect on C'4, which, when treated with C'1a in free solution, is also rapidly converted to inactive C'4 (16). Very small amounts of C'1 may be detected by these two methods, as the effect of the enzyme on its substrates is striking. One microgram of C'1 esterase, for instance, was found to inactivate 4,000 μg of purified C'4 in 20 minutes at 37°C (26). The loss of C'4 activity may readily be determined by standard technics.

Another manifestation of the interaction of complement with cell-bound antigen antibody complexes is the formation on the cell surface of the C'4,2a complex, or C'3 convertase. This enzyme catalyzes uptake of C'3 to the cell surface, which may be detected by one of the following three methods. Cells carrying the third component of complement on their surface exhibit immune adherence activity (28, 32); that is, they tend to adhere to other cells such as human erythrocytes which may serve as indicator cells. Utilizing radioactively labeled, purified C'3 it was found that at least 60 C'3 molecules per cell are required to give a positive immune adherence reaction.[5] A strongly positive reaction, however, requires several hundred bound C'3 molecules per cell. Approximately 150–200 molecules of bound C'3 per cell are necessary in order that these cells give a positive agglutination reaction with a monospecific antiserum to C'3. Thus, the agglutination reaction using anti-C'3 antibody appears to be somewhat less sensitive than the immune adherence reaction. A very sensitive indicator for the presence of cell-bound C'4,2a complex is uptake of radioactive C'3. Labeling is accomplished without loss of cytolytic activity using ^{125}I, and a few molecules of cell-bound C'3 may be detected utilizing this tool provided that the cell sample is not too small (24). The sensitivity of the latter method may be increased if an excess of radioactively labeled C'3 is used, since under these conditions each single cell-bound C'4,2a complex is capable of catalyzing uptake of as many as several hundred C'3 molecules. The limiting factor in the use of the C'4,2a complex as an indicator for cell-bound antigen-antibody complexes is its restricted life span, which, however, can be much enhanced by use of chemically modified C'2.

It has been known for some time that C'2 can be inactivated by para-chloromercuribenzoate (*p*-CMB) (13). In exploring the effects of other sulfhydryl reagents, it was found that iodine can greatly enhance the hemolytic activity of human C'2 (34). By effective molecule titration, the hemolytic activity of C'2 was 13-fold greater following treatment with a critical dose of iodine. The increased activity of C'2 could be explained on the basis of an increased enzymatic activity of the C'4,2a complex prepared with iodine-treated C'2. In addition, it was noted that the complex was up to 15 times more stable than when prepared with untreated C'2. Effective iodine treatment requires at least partially purified C'2, although treatment of diluted whole human serum with iodine results in some enhancement of C'2 hemolytic activity. In exploring the nature of the iodine effect, it was found that iodine-treated C'2 was no longer susceptible to the inhibitory action of *p*-CMB, and this finding suggested that *p*-CMB and iodine affected the same group in the C'2 molecule, possibly a sulfhydryl group. Both substitution and oxidation of a sulfhydryl group by iodine were considered as possible explanations. However, enhancement of C'2 hemolytic activity did not correlate with iodine uptake by C'2 and, furthermore, since the effect of iodine on the C'2 activity could be reversed by the reducing compound, sodium dithionite, it was considered most probable that iodine achieved the enhancing effect by oxidation of a sulfhydryl group. The iodine-modified C'2 is, therefore, referred to as ^{oxy}C'2. Thus far, the use of ^{oxy}C'2 has greatly facilitated in this laboratory the work on the formation of C'3 convertase from C'2 and C'4 and on the effect of the enzyme on the C'3 molecule. It may be expected that the use of ^{oxy}C'2 may also facilitate the detection of cell surface antigens by antibody and complement.

[5] N. R. Cooper and H. J. Müller-Eberhard, unpublished observations.

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