The Possible Use of Complement for the Detection of Cell Surface Antigens\textsuperscript{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.\textsuperscript{2} Of the eleven human complement proteins, seven have thus far been obtained in a high degree of molecular homogeneity \[\text{C}'1q (25), \text{C}'1s (8), \text{C}'2 (17, 33, 35), \text{C}'3 (30), \text{C}'4 (23), \text{C}'5 (30) \text{and C}'9\textsuperscript{4}\]. 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; how

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3 H. J. Müller-Eberhard, M. A. Calcott, and H. M. Grey, Interaction between \text{C}'1q and \gamma\text{-Globulins of Different Heavy Chain Subtypes, manuscript in preparation.}

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Table 1

<table>
<thead>
<tr>
<th>Properties</th>
<th>C1q</th>
<th>C1r</th>
<th>C1s</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
<th>C7</th>
<th>C8</th>
<th>C9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum conc. (µg/ml)</td>
<td>100-200</td>
<td>22</td>
<td>10</td>
<td>1200</td>
<td>430</td>
<td>75</td>
<td>8</td>
<td>5-6</td>
<td>5-6</td>
<td>8</td>
<td>4.5</td>
</tr>
<tr>
<td>Sedimentation rate (S)</td>
<td>11.1</td>
<td>7</td>
<td>4</td>
<td>5.5</td>
<td>9.5</td>
<td>10</td>
<td>8.7</td>
<td>8</td>
<td>8</td>
<td>4.5</td>
<td>79,000</td>
</tr>
<tr>
<td>Approx. mol. wt.</td>
<td>400,000</td>
<td>115,000</td>
<td>240,000</td>
<td>220,000</td>
<td>120,000</td>
<td>8,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrophoretic</td>
<td>γ2</td>
<td>βα1</td>
<td>βα2</td>
<td>βα3</td>
<td>β1</td>
<td>β1</td>
<td>β2</td>
<td>β2</td>
<td>γ1</td>
<td>α</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate conc. (%)</td>
<td>17</td>
<td>2.7</td>
<td>14</td>
<td>19</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive SH</td>
<td>1 or more</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</table>

Properties of human complement components.

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 (2). This may be demonstrated by studying the reactivity of typed myeloma proteins with isolated C1q (3). While γG1- and γG3-globulins exhibited a pronounced affinity for C1q, γG2-globulins reacted poorly or not at all, and γG3-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 C1, and the second is the formation on the cell surface of C3 convertase. There are at least three methods for the detection of each of these two reactions.

Binding and activation of C1 may be detected by C1 transfer, as pointed out by Borsos and Rapp (1). Activated C1 (C1a) tends to dissociate from its complexes with sensitized cells, the equilibrium between cell-bound C1a and free C1a depending on the ionic strength of the buffer. While at physiologic ionic strength a considerable proportion of C1a is unbound, most of the C1a 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 C1. The cells are washed at low ionic strength to remove all unbound C1 and are then mixed, at physiologic ionic strength, with sensitized sheep erythrocytes containing the fourth component of complement to allow transfer of C1a to these indicator cells. Assay of C1a on the surface of sensitized sheep erythrocytes is then carried out according to standard procedures. It is said that one molecule of C1a per cell is sufficient to cause lysis of 63% of the cells present under optimal conditions. Since C1a is an enzyme and since C2 and C4 are its two natural substrates, it is obvious that activation of C1 may also be detected by its effect on the two substrates. If purified C2 or a serum fraction containing C2 is incubated with C1a, C2 is rapidly inactivated (16, 37) by cleavage of the molecule into at least two fragments (35, 38). The degree of reduction...
of C2 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 C1a may be measured by its effect on C4, which, when treated with C1a in free solution, is also rapidly converted to inactive C4 (16). Very small amounts of C1 may be detected by these two methods, as the effect of the enzyme on its substrates is striking. One microgram of C1 esterase, for instance, was found to inactivate 4,000 µg of purified C4 in 20 minutes at 37°C (26). The loss of C4 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 C4,2a complex, or C3 convertase. This enzyme catalyzes uptake of C3 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 C3 it was found that at least 60 C3 molecules per cell are required to give a positive immune adherence reaction.6 A strongly positive reaction, however, requires several hundred bound C3 molecules per cell. Approximately 150–200 molecules of bound C3 per cell are necessary in order that these cells give a positive agglutination reaction with a monospecific antiserum to C3. Thus, the agglutination reaction using anti-C3 antibody appears to be somewhat less sensitive than the immune adherence reaction. A very sensitive indicator for the presence of cell-bound C4,2a complex is uptake of radioactive C3. Labeling is accomplished without loss of cytolytic activity using 125I, and a few molecules of cell-bound C3 may be detected utilizing this probe 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 C3 is used, since under these conditions each single cell-bound C4,2a complex is capable of catalyzing uptake of as many as several hundred C3 molecules. The limiting factor in the use of the C4,2a 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 C2.

It has been known for some time that C2 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 C2 (34). By effective molecule titration, the hemolytic activity of C2 was 13-fold greater following treatment with a critical dose of iodine. The increased activity of C2 could be explained on the basis of an increased enzymatic activity of the C4,2a complex prepared with iodine-treated C2. In addition, it was noted that the complex was up to 15 times more stable than when prepared with untreated C2. Effective iodine treatment requires at least partially purified C2, although treatment of diluted whole human serum with iodine results in some enhancement of C2 hemolytic activity. In exploring the nature of the iodine effect, it was found that iodine-treated C2 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 C2 molecule, possibly a sulfhydryl group. Both substitution and oxidation of a sulfhydryl group by iodine were considered as possible explanations. However, enhancement of C2 hemolytic activity did not correlate with iodine uptake by C2 and, furthermore, since the effect of iodine on the C2 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 C2 is, therefore, referred to as δC2. Thus far, the use of δC2 has greatly facilitated in this laboratory the work on the formation of C3 convertase from C2 and C4 and on the effect of the enzyme on the C3 molecule. It may be expected that the use of δC2 may also facilitate the detection of cell surface antigens by antibody and complement.

REFERENCES

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