The Emerging Clinical Usefulness of Complement Measurements*

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Not many years ago the main purpose of “complement” seemed to be to drill holes in sheep erythrocytes. In the classic experiment which was part of every medical student’s microbiology laboratory experience, a magic stuff called complement, somewhat mysteriously obtained from guinea pigs, was either “fixed” or not “fixed” and the sheep cells either not lysed or lysed accordingly. That was about all there was to know about complement, and all one needed to know.

Today the term “complement” embodies a group of plasma proteins which react in a complex sequence to mediate a variety of inflammatory effects, including changes in vascular permeability, the attraction of polymorphonuclear or mononuclear leukocytes, the enhancement of phagocytosis, and damage to cell membranes and osmotic lysis such as the sheep erythrocyte suffered in the complement fixation test. These complement proteins rival the intrinsic coagulation scheme in complexity and resemble it in mechanism, that is, the complement proteins normally circulate in the plasma in an inactive or precursor form, and when appropriately stimulated, usually during an immunologic reaction, become transformed into active enzymes, or proteases. These proteases act upon their natural substrates (other members of the complement system) in an orderly and predetermined sequence of limited proteolytic reactions which are often compared to a “cascade” or “waterfall.” Cleavage of one component leads to the activation of the next component, and so forth. Natural inhibitors or inactivators also present in plasma serve to modulate or damp this cascade system and prevent its getting out of control.

In fact, congenital deficiency of one of these inhibitors, the C1 Inhibitor, leads to uncontrolled activation of the system and recurrent swelling of the subepithelial tissues of the skin, respiratory and gastrointestinal tracts.2

Biochemical Pathways.

Two pathways for activation, the classic and alternative (properdin), initiate the terminal attack sequence which elaborates most of the biologic activities associated with complement. The classic pathway is activated by immune complexes containing IgG or IgM immunoglobulins and their associated antigens. The properdin pathway is activated by certain kinds of repeating polysaccharides such as pneumococcal polysaccharide, or the bacterial lipopolysaccharide of gram-negative endotoxin; immune complexes containing IgA may also activate the properdin system.

Regardless of which pathway is activated, both result in the cleavage of C3 and C5. Peptides released from these components, C3a and C5a, are anaphylatoxins capable of releasing histamine from mast cells and thereby influencing local vascular permeability. C5a also releases lysosomal enzymes and presumably other granular contents from polymorphonuclear leukocytes. Both C3a and C5a have chemotactic activity as well, the latter being more active in most systems; the trimolecular complex formed from C5, C6, and C7 is also chemotactic. Immune complexes to which complement, especially

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C3b, has become bound adhere to polymorphonuclear neutrophils, mononuclear cells and B-lymphocytes. Although the functional significance of these binding phenomena is not yet entirely clear, enhanced phagocytosis by mononuclear or polymorphonuclear cells is certainly one consequence. Formation of a multimolecular complex involving C5, C6, C7, C8, and C9 leads to the membrane damage and osmotic lysis which have become the hallmarks of complement activation.

Metabolism of Complement Proteins.

One result of the proteolysis of the complement proteins during their activation is that they subsequently become recognizable as "altered" or "foreign" by the body and are rapidly cleared from the circulation. Although some compensatory increases in synthesis may occur, the result is usually a fall in plasma level. Thus, an ongoing immunologic event (or disease) which is activating the complement system in vivo may be manifested as a fall in the serum or plasma level of one or more of the complement proteins. In reverse fashion, as the complement-activating stimulus or disease abates, this may be paralleled by a return towards normal in the complement levels.

Determinations of Complement in the Clinical Pathology Laboratory.

In principle, there are two ways of measuring complement: (1) by its activity in the reaction it catalyzes, for example, the total hemolytic complement or CH50, which measures the result of the interaction of all nine of the classic complement components or (2) by its antigenicity, as a protein in an immunassay which takes advantage of the complement protein's capacity to react with monospecific antibody directed against it. In practice, although total hemolytic complement or CH50 determinations are available in some institutions, immunassays (usually radial immunodiffusion) are most frequently available. Materials for these are offered in the form of kits for use in the clinical pathology laboratory by a number of commercial suppliers.

A few comments are in order about the radial immunodiffusion determinations for complement components available in most hospitals.

1) Radial immunodiffusion, by its very nature, is not nearly as precise a determination as most physicians have come to expect from clinical laboratories. Under the best conditions, the coefficient of variation of the test is likely to be 8% or greater, more than twice that of commonly available clinical chemistry or hematologic procedures. Thus in interpreting the results of the test, the physician must take into account this reduced precision, for example, a "fall" in C3 level from 145 to 130 mg/100 ml from one day to the next may reflect only laboratory variation.

2) There are no widely available standards, so that considerable variation in absolute values obtained by kits from different suppliers or even in lots of kits from the same supplier may be observed. This should not be a problem if results are referred to a normal range collected at the institution in which the test is being performed, and if appropriate internal standards, maintained at the institution, are assayed in parallel with the test samples. If the "normal ranges" provided with the kits are accepted as verbatim, and if independent checks of the performance of the kits are neglected, then unreliable data may result.

3) As for other plasma proteins, the range of normal for complement proteins is quite broad, usually in the vicinity of ± 50% of the mean value for the population. Thus changes in levels in a single patient over a period of time may often be more helpful and easier to interpret than are comparisons with some absolute range of normal. For example, a patient with suspect systemic lupus erythematosus whose C4 level fell from 70 mg/100 ml to 30 mg/100 ml within two weeks might be cause for alarm, even though the latter value was still "within the range of normal."

4) The most widely available test for a complement component (C3) is not necessarily the most desirable. Its availability is directly related to the fact that C3 is by far the most plentiful of the complement components, the easiest to purify, and therefore the easiest to make antibody against for use in a radioimmunoassay. Measurements of C3 were therefore widely available from commercial sources several years in advance of measurements of other components. Most workers would agree today, however, that measurements of C4 are likely to be more sensitive to minor episodes of in vivo complement activation, and that a good "routine" complement screen would include measurements of C4, C3, and possibly CH50.
5) The immunoassays do not distinguish between native protein and that which has participated in complement activation and lost its activity but not its immunogenicity. They are reliable, therefore, only in instances in which substantial amounts of cleaved, inactive protein would not be expected. Altered complement proteins are cleared within a few hours from the plasma space, and the finding of altered inactive protein in plasma requires special techniques to detect the small amounts which are present. In contrast, however, altered inactive protein may persist for much longer times in joint spaces or pleural spaces, so that radial immunodiffusion determinations of C3 or C4 in synovial or pleural fluid are of very little value. Recent studies have shown that measurements of C4 in cerebrospinal fluid are similarly of little diagnostic value with respect to the presence or absence of central nervous system involvement in systemic lupus erythematosus.

Clinical Significance.

Given the knowledge that the complement system may be activated in vivo by immunologic diseases, and that simple and reliable methods for measuring complement levels are now widely available, how can the practitioner best use this information? What are the diseases in which complement measurements are likely to be of help, either in diagnosis or in following the course of the patient? The answer is simple: any disease in which the physician suspects that circulating immune complexes may be playing a pathogenetic role. A few of them are listed in the Table.

Systemic lupus erythematosus is the disease which is perhaps most commonly associated with hypocomplementemia, but it is well to bear in mind that other diseases in which circulating antigen-antibody complexes may be found may also give rise to hypocomplementemia. Among these would be included any cause of "chronic antigenemia," for example, subacute bacterial endocarditis, hepatitis B surface antigenemia, infected atrioventricular shunts, recurrent gram-negative sepsis, recurrent viremias such as dengue hemorrhagic fever, or recurrent parasitemia, such as falciparum malaria. Other diseases of unknown etiology may be associated with hypocomplementemia, such as essential mixed cryoglobulinemia, or certain kinds of nephritis (which in contrast to all the diseases mentioned above which are likely to have low C4, and sometimes low C3, usually have only low C3, suggesting direct alternative pathway activation). The symptom of "angioedema" is infrequently associated with hypocomplementemia, but a screening test should be done for C4, which is low in almost all cases of hereditary angioedema, especially since very effective treatment for this disease is now available.

In most instances in which hypocomplementemia is found in association with the disease, improvements in complement levels are often early and reliable indices that the disease is ameliorating either spontaneously or as a result of therapy.

Thus, by applying his understanding of "complement fixation," obtained in the classical microbiology laboratory experiment, to human diseases in which "complement fixation" appears to be occurring in vivo, today's physician has achieved a useful diagnostic tool and therapeutic index in the measurement of complement components in disease.

TABLE

Diseases in which Complement Determinations May Provide Useful Diagnostic or Therapeutic Information

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<th>Disease</th>
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<tbody>
<tr>
<td>Systemic lupus erythematosus</td>
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<td>Rheumatoid arthritis (with systemic vasculitis)</td>
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<tr>
<td>Hypersensitivity angiitis</td>
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<td>Angioedema</td>
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<td>Glomerulonephritis</td>
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<td>Subacute bacterial endocarditis</td>
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<td>Hepatitis</td>
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<td>Essential mixed cryoglobulinemia</td>
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