The complement system is one of the main defense mechanisms of innate immunity. Deficiencies of complement components are generally associated with an increased susceptibility to a wide range of infections. In addition, deficiencies of certain complement components predispose individuals to immune complex diseases, such as vasculitis and glomerulonephritis. Recent studies have linked deficiencies of the complement system to a diminished acquired immune response and to an inability to clear apoptotic cells, and subsequently with autoimmunity. Moreover, recent studies suggest that some complement deficiencies contribute to the development of atherosclerosis.

The complement activation pathways

The complement system is composed of at least 30 proteins that are present in the circulation or in various body fluids in a non-activated (pro-enzymatic) form. To initiate biological activity from the complement system, there are three pathways of activation. The most well known of these is the classical pathway. This pathway is initiated by the interaction of C1q, the recognition molecule of the classical pathway, with, for example, antigen-antibody complexes. Most frequently these complexes contain IgG or IgM antibodies, and negatively charged antigens. C1q binding leads to activation of its associated serine proteases C1r and C1s, followed by subsequent activation of C4 and C2, resulting in the formation of the classical pathway C3 convertase, C4b2a. This enzyme cleaves C3 into C3b and C3a. C3b can attach itself covalently to the activator, and following amplification of C3 cleavage, can recruit the terminal sequence of complement. Subsequently the highly biologically active anaphylatoxin C5a is released and the terminal complex of C5b-C9, also called the membrane attack complex (MAC), is formed which causes bacteriolysis, cytolysis, apoptosis, as well as activation of the attacked cell.

In addition to the classical pathway, activation of complement can also occur via the alternative pathway. This pathway is mainly activated by bacteria and yeast, but also by immune complexes composed of IgA or IgG. During this pathway, the complement components B, D and properdin, together with C3, stimulate the generation of an alternative pathway C3 convertase (C3bBbP) and subsequent cleavage of C3 and recruitment of the effector pathway C5b-C9.

Finally, a third pathway, which is mainly antibody independent, is initiated by the recognition of specific patterns of carbohydrate moieties by a serum protein, mannos-binding lectin (MBL). MBL is associated with a number of proteases called MBL-associated serine proteases (MASPs), which are converted from a proenzymatic state to an activated form when MBL binds to its activator. Activated MASPs then cleaves C4 and C2, again leading to the formation of C4b2a, a C3 convertase identical to the one generated following activation of C1 by the classical pathway. Activation of C3, regardless of which pathway of complement is activated, is one of the most important steps in complement biology. Deposition of C3b on activator surfaces allows phagocytic cells and other immune cells to recognise complement activators (i.e. antigens, bacteria, yeast, and viruses) in a more efficient fashion, and therefore it is responsible for the effective clearance of unwanted material. This explains why deficiencies in any of the activator pathways, or C3 itself, may predispose individuals to various types of infections, to an inefficient clearance of immune complexes, or to an aberrant immune response.

Quantitative assays to determine the activities of the three pathways of complement

In recent years, adequate monoclonal and polyclonal antibodies have become available for the quantification of most complement components. Single component assays are usually carried out in experienced laboratories by a number of existing techniques, including radial immunodiffusion, ELISA, radioimmunoassay or nephelometry. However, for diagnostic purposes, measurement of single complement components in addition to performing assays that quantify the overall activity of the three pathways of complement, is not generally useful. In addition, the measurement of a few selected components, as is done for C3 and C4 in most clinical laboratories, is not the most efficient way to use scarce resources.

For several decades complementologists have sought assays that quantify, in a reproducible way, the biological activity of the known pathways of complement. For many years, sheep erythrocytes coated with rabbit antibodies have been used to assess the activity of the classical pathway. Antibody-sensitised sheep erythrocytes (EA) are incubated with dilutions of diagnostic serum samples or a standard serum, and the activity of the classical pathway (CH50) is assessed based on the degree of cytolysis induced by a certain dilution of sample relative to the standard. This assay is quite satisfactory, but is difficult to standardise between laboratories.

Complement defects can be identified from the results of the three assays performed in parallel. Deficiencies or abnormalities in the complement system that can explain the possible outcomes of the three complement assays are described in Table 1. Thereafter, measurements of specific components can be performed following consultation with a clinical immunologist, a clinical pathologist or a complement expert.

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Figure 1. Brief overview of the principle of the Wielisa ELISA test for complement activity. The assay is based on three principle steps: 1) three different types of wells, each coated with a specific component, 2) an incubation step specific for each pathway and where interference from the two other pathways is blocked, 3) a common detection step in which the formed “Membrane Attack Complex” is detected.

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