Review article

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THE COMPLEMENT SYSTEM: PATHWAYS OF ACTIVATIONS AND FUNCTIONS

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The complement system plays an important role in host defense. It is composed of series of plasma proteins which, when activated in a sequential reaction, unleashes a powerful destructive activity towards invading pathogens. Three major pathways exist in the activation of complement: the classical pathway, which is initiated by antibody-antigen binding, the alternative pathway, which is activated by a susceptible foreign surface, and the lectin pathways, which can be triggered by manann-binding lectins.

The complement system consists of serum and cell surface proteins that interact with one another and with other molecules of the immune system in a highly regulated manner. Complement proteins are plasma proteins that are normally inactive and they are activated only under particular conditions to generate products that mediate various effector functions of the complement. *Acta Medica Medianae 2017;56(1):50-55.*

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Introduction

Complement is a part of the innate immune system. It is now known that complement is a system of more than 30 different plasma and membrane proteins. It has three physiological activities: defending against pyogenic bacterial infection, bridging innate and adaptive immunity during the classical pathway of complement activation, disposing of immune complexes and the products of inflammatory injury. Complement may be activated by three different mechanisms, known as the classical, alternative, and lectin pathways (1).

Classical pathways of complement activation

H The classical pathway is initiated by binding of the complement protein C1 to the C_H2 domains of IgG or C_H3 domains of IgM molecules that have bound antigen.

C1 (750 kDa) is a large, multimeric protein complex composed of C1q, C1r, and C1s subunits. C1q subunit, molecular weight of 460 kDa, is a hexamer of three pairs of chains (22, 23 and 24 kDa), made up of an umbrella-like radial array. The globular heads at the end of each chain are the contact regions for immunoglobulin. The C1q molecule must bind to two Ig heavy chains to be activated.

C1r (85 kDa, dimer) and C1s (85 kDa, dimer) are serine esterases that function as a tetramer containing two molecules of each. Binding of two or more of the globular heads of C1q to Fc regions of IgG or IgM leads to enzymatic activation of the associated C1r, which cleaves and activates C1s. Activated C1s cleaves the next protein in the cascade, C4 component into larger C4b and smaller C4a fragments (2,3).

C2 (102 kDa, monomer), the next component, is then cleaved into larger C2a fragment, and smaller C2b fragment, by C1s. C2a attaches noncovalently to C4b, forming a C4aC2a complex that is termed as a classical pathway C3 convertase (3).

C3 component (185 kDa) consists of α (110 kDa) and β (75 kDa) subunits. Cleavage of C3, by C3 convertase, results in the removal of a small C3a fragment, which leaves C3b. A larger fragment, C3b, also contains a thioester bond, therefore, C3b can also be covalently attached to the surface of the antigenic target, to immunoglobulin. Once C3b is deposited, it can bind factor B and generate more C3 convertase by the alternative pathway. Some of the C3b molecules generated by the classical pathway C3 convertase bind to the complex C4bC2a. This complex functions as the classical pathway C5 convertase which cleaves C5 into the C5a and C5b If these bonds are not formed, the C3b remains in the fluid phase, its

thioester is quickly hydrolyzed, and it becomes inactive and complement activation stops (4).

Alternative pathway of complement activation

The alternative pathway differs from the classical pathway by its mechanism of initial activation. Some substances, such as polysaccharides, endotoxins, virally infected cells (influenza, Epstein-Barr virus), yeast cells wall extracts (zymosan) and some immunoglobulin light chains can serve to promote alternative pathway activation.

Under certain circumstances, the alternative pathway has the capacity to autoactivate because normally C3 in plasma is being continuously cleaved at a low rate to generate C3b in a process that is marked as "C3 tickover" (5). An internal thioester bond in C3 becomes unstable when the molecule is cleaved, and it reacts with the amino or hydroxyl groups of cell surface proteins or polysaccharides to form amide or ester bonds. In this case, the complement is activated by alternative pathway. If these bonds are not formed, C3b remains in the fluid phase, its thioester is quickly hydrolyzed, and it becomes inactive and complement activation stops.

After binding to the surface of microorganisms, C3b binds a plasma protein called factor B. After it is bound, factor B is cleaved by a plasma serine protease called factor D to generate a smaller Ba fragment and larger Bb fragment (remains attached to C3b). The C3bBb complex is the alternative pathway C3 convertase. In addition, another protein of the alternative pathway, called properidin, can bind to and stabilize the C3bBb complex. Some of the C3b molecules generated by the alternative pathway C3 convertase bind to the convertase itself. This results in the formation of a C3bBbC3b complex which functions as the alternative pathway C5 convertase to cleave C5 and initiate the late steps of complement activation (2, 6).

Lectin pathway of complement activation

Lectin pathway of complement activation is induced by binding of manose residues on the surface of mocroorganisms to soluble complex that consists of mannose-binding lectin (MBL), enzyme inert protein molecular weight of 19 kDa and three specific serin esterase (mannose-binding lectin associated serin proteases, MASPs 1, 2, and 3). This complex is structurally similar to C1 component of the complement (7-9).

MBLs consist of three polypeptide chains, all containing collagen-like domain and the carbohydrate recognition domain (CRD). That binds specifically to mannose or N-acetylglucosamine, placed at the surface of microorganisms. Afterwards, the complex MBL-MASPs can activate C4 and C2 components of the complement. MASP1 can also directly activate C3 component of the complement (1, 10-12).

Late steps of complement activation

Common to all pathways of complement activation is the formation in the certain moment of C5 convertase (serin esterase) that degrades C5 (190 kDa, dimmer) component of the complement to the smaller C5a, and the larger, C5b fragment.

One interesting difference between the early activation pathway and the later components of complement (after C5) is the absence of proteolitic activity in the later components. C6 through C9 assembly is the product of conformational changes that exposes new reactive sites on each new complement component.

C5b fragment remains bound to the cell surface. The remaining components of the complement cascade, C6, C7, C8, and C9, are structurally related proteins without enzymatic activity. C5b transiently maintains a conformation cascade, C6, and the stable C5bC6 complex remains loosely associated to the cell membrane until it binds a single C7 molecule.

The C7 component of the resulting C5bC6C7 complex is hidrofobic, and it inserts into lipid bilayer of cell membranes, where it becomes a high-affinity receptor for one C8 molecule (155 kDa, trimer). Activation of this complex is complete after binding C9 component (79 kDa, monomer). C9 is a serum protein that polymerizes at the site of the bound C5bC6C7C8 complex and forms pores in plasma membranes. C9 is structurally homologous to perforin (3).

Complex C5bC6C7C8C9 is called membrane attack complex (MAC) or terminal complement complex (TCC). MAC leads to the lysis of cells, by forming on the cell membrane pores with diameter about 1 nm that form channels allowing free movement of water and ions (13).

Functions of complement

The principal effector functions of the complement system in innate immunity and specific humoral imunity are to promote phagocytosis of microbes on which complement is activated, to stimulate inflammation, and to induce the lysis of these microbes. In addition, products of complement activation provide "second signals" for the activation of B lymphocytes and the production of antibodies (3).

The proteolytic complement fragments C5a, C4a and C3a are called anaphylatoxins and induce acute inflammation by acting on mast cells and neutrophils. All three peptides bind to mast cells and induce degranulation, with the release of vasoactive mediators such as histamine. In neutrophils, C5a stimulates chemotaxis, film adhesion to endothelial cells, and at high doses, stimulation of the respiratory burst and production of reactive oxygen intermediates. In addition, C5a may act

directly on vascular endothelial cells and lead to increased vascular permeability and the expression of P-selectin, which promotes neutrophil binding. C5a is the most potent mediator of mast degranulation (14).

Complement receptors

Type 1 complement receptor (CR1, CD35) is a member of a large family of C3- and C4-binding proteins known as the regulators of complement activation (RCA). CR1 is expressed on human erythrocytes, monocytes, polimorphonuclear cells, B and T lymphocytes, eosinophils and follicular dendritic cells.

Soluble forms of CR1 in serum and urine have been described.

The major form of CR1 is a 190 kDa protein, although less frequent allelic forms of 160, 220 and 250 kDa have also been described (15).

The major role of CR1 on erythrocytes is the trapping of circulating immune complexes and the subsequent transfer of these complexes to Kupffer cells and macrophages in the liver and spleen.

CR1 promotes phagocytosis by monocytes and neytrophils of targets that are also coated with Ig, thereby functionally interacting with Fc receptors (16).

Type 2 complement receptor (CR2, CD21) is a protein with molecular weight of 145 kDa. CR2 is present on B lymphocytes, follicular dendritic cells, a small subset of T lymphocytes, thymocytes, epithelial cells, basophils, and keratinocytes. It specifically binds the cleavage products of C3b: C3b and C3dg. It also serves as the receptor for the Epstein-Barr virus (15, 17).

The major role of CR2 on B lymphocytes is to serve as a costimulator that acts by amplifying antigen-induced B cell activation through surface IgM. CR2 coassociates on the cell membrane with a B-lymphocyte-specific protein designated CD19, CD81, Leu13, MHC II and γ-glutamyl transpeptidase (GGT). This complex delivers signals to B cells that enhance the responses of B cells to antigen. This complex also regulates the homotypic adhesion of cells. Coligation of CD19 or CR2 strongly promotes B-limphocyte activation (18).

Type 3 complement receptor (CR3, Mac-1, CD11b/CD18) is a heterodimer (165 kDa) and belongs to the β2-integrin family. CR3 consists of an α chain (CD11b) noncovalently linked to the β chains (CD18). CR3 is expressed primarily by neutrophils, monocytes, follicular dendritic cell, Kupffer cells, and NK cells. CR3 is a receptor for C3 and C3 fragments. The affinity for the C3 fragments varies, with C3bi>C3b>C3d. CR3 plays an important role in the phagocitosis of C3bi coated particles (19).

Type 4 complement receptor (CR4, CD11c/CD18) is an another member of β2-integrin family. CR4 is expressed by dendritic cells, neutrophyls and NK cells. CR4 also binds C3bi >C3b but its biologic activities are similar to CR3 (19).

C1q receptor is expressed on macrophages, endothelial cells, fibroblasts, platelets, epithelial cells and leukocytes. Although four receptors for C1q have been described, only one of them appears to function as a typical transmembrane receptor. This is C1qRp (C1q receptor-related protein). C1qRp is a transmembrane protein expressed on monocytes, macrophages, platelets and endothelial cells. The receptor interacts with C1q collagen-like tails and MBL. C1qRp can enhance CR1 mediated phagocytosis (20, 21).

C5a receptor (CD88) is an approximately 50 kDa transmembrane-spanning protein expressed on mast cells, PMN, macrophages, monocytes, T cells, eosinophils, hepatocytes, vascular endothelium, astrocytes, bronchial, and alveolar epithelial cells (22, 23).

C3a receptor is transmembrane-spanning protein (60 kDa) expressed on PMN, monocytes, and basophils (23).

Regulation of the complement system

Mammals possess several mechanisms to protect bystander cells of the deleterious effects of autologous complement activation. These protective mechanisms involve serum and membrane molecules (complement regulatory proteins), which specifically recognize and inactivate components of the classical, alternative or lectin pathways of complement activation. They protect autologous cells from complement damage either by restricting C3 cleavage and its deposition or by inhibiting MAC formation. Several membrane-bound complement regulators in humans have been characterized: decay-accelerating factor (DAF, CD55), membrane cofactor protein (MCP, CD46), CD59, complement receptors 1 (CR1, CD35) and 2 (CR2, CD21). These molecules belong to a family of structurally and genetically related proteins named regulators of complement activation (RCA) and contain homologous repetitive domains (short consensus repeats, SCR) which are responsible for the protective function against autologous complement activation (24, 25).

The complement proteins interact with other endogenous molecules to keep microbes from entering the parenteral tissues. One of the mucosal proteins known to interact with the early complement components of both the classical and the lectin pathway is the salivary scavenger and agglutinin (SALSA). SALSA is also known as deleted in malignant brain tumors 1 and gp340. It is found both attached to the epithelium and secreted into the surrounding fluids of most mucosal surfaces. SALSA has been shown to bind directly to C1q, mannose-binding lectin, and the ficolins. Through these interactions, SALSA regulates the activation of the complement system (26-28).

Complement system in disease

The complement system may be involved in different human disease.

Defficiencies in any components of complement (C1q, C1r, C2, C3, C4) may lead to abnormal patterns of complement activation. If normally generated immune complexes are not cleared from the circulation, they may be deposited in blood vessel walls and tissues, where they activate leukocytes and produce local inflammation (29).

C2 and C4 deficiencies are not usually associated with increased susceptibility to infections, which suggests that the alternative pathway is adequate for host defense against most microbes. Deficiency of C3 is associated with frequent serious pyogenic bacterial infections and this illustrates the importance of C3 for opsonization and phagocytosis. Deficiencies in components of the alternative pathway result in increased susceptibility to infection with pyogenic bacteria.

Deficiencies in the terminal complement components (C5, C6, C7, C8, C9) have also been described. Interestingly, the only consistent clinical problem in these patients is infection by Neisseria bacteria.

Deficiencies in CR3 and CR4 both result from mutations in the genes. This disorder is characterized by inadequate adherence of neutrophils and perhaps by impaired C3b dependent phagocytosis of bacteria (17).

Even when it is properly regulated and appropriately activated, the complement system can cause significant tissue damage. In some situations, complement activation is associated with intravascular thrombosis and can lead to ischemic injury to tissues. For instance, anti-endothelial antibodies against vascularized organ transplants may bind to vascular endothelium and activate complement, thereby leading to the generation of MAC and damage to the endothelial surface, which favors coagulation. There is also evidence that some of late complement proteins may activate prothrombinases (30).

Systemic vasculitis and glomerulonephritis result from the deposition of immune complexes in the walls of vessels and kidney glomeruli. Complement activated by the immunoglobulin in these deposited immune complexes initiates the acute inflammatory responses that destroy vessels walls or glomeruli (31).

Conclusion

During an infection, complement leads to inflammation, opsonization, phagocytosis, and destruction of the pathogen, and ultimately results in activation of the adaptive immune response. Both inefficient and overstimulation of the complement can be detrimental for the host and are associated with increased susceptibility to infections or non-infectious diseases, including autoimmunity, chronic inflammation, graft rejection, thrombotic microangiopathy.

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Sistem komplementa: putevi aktivacije i funkcija

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Sistem komplementa ima značajnu ulogu u odbrani domaćina od patogena. Sastoji se od niza proteina plazme koji se aktiviraju u kaskadnom procesu, dovodeći do oštećenja patogena. Opisana su tri puta aktivacije sistema komplementa: klasičan (aktivira se vezivanjem C1q komponente komplementa za kompleks antigen-antitelo), alternativni (aktivira se direktnim vezivanjem C3b komponente komplementa za površinske molekule mikroorganizama) i lektinski (indukovan je vezivanjem manoza vezućeg proteina za manozne rezidue na površini mikroorganizama).

Najvažnija uloga sistema komplementa u urođenom i specifičnom imunitetu je stimulisanje fagocitoze, liza mikroorganizama i pospešivanje inflamacije. Komplement ima ključnu ulogu i u uklanjanju imunskih kompleksa. *Acta Medica Medianae 2017;56(1):50-55.*

Ključne reči: *sistem komplementa, aktivacija, klasični, alternativni i lektinski put*