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Complement in clinical medicine: Clinical trials, case reports and therapy monitoring

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ABSTRACT

Research during past decades made it evident that complement is involved in more tasks than fighting infections, but has important roles in other immune surveillance and housekeeping functions. If the balance between complement activation and regulation is out of tune, however, complement can quickly turn against the host and contribute to adverse processes that result in various clinical conditions. Whereas clinical awareness was initially focused on complement deficiencies, excessive activation and insufficient regulation are frequently the dominant factors in complement-related disorders. The individual complement profile of a patient often determines the course and severity of the disease, and the pathophysiological involvement of complement may be highly diverse. As a consequence, complement assays have evolved as essential tools not only in initial diagnosis but also for following disease progression and for monitoring complement-targeted therapies, which become increasingly available in routine clinical use. We herein review the current state of complement-directed drug candidates in clinical evaluation and provide an overview of extended indications considered for the FDA-approved inhibitor eculizumab. Furthermore we review the literature describing cases reports and case series where eculizumab has been used "off-label". Finally, we give a summary of the currently available tests to measure complement profiles and discuss their suitability in diagnostics and treatment monitoring. With complement finally entering the clinical arena, there are intriguing opportunities for treating complement-mediated diseases. However, this progress also requires a new awareness about complement pathophysiology, adequate diagnostic tools and suitable treatment options among clinicians treating patients with such disorders.

1. The changing landscape of complement in disease and therapy

The past few decades have led to a profound shift in our perception of the human complement system. Commonly known as an innate immunity segment of the host defense system that protects our bodies from invading microbes and other threats, it has become increasingly evident that complement has important roles in other immune surveillance and housekeeping tasks but also contributes to a wide and diverse range of clinical disorders (Ricklin et al., 2010; Ricklin et al., 2016). The reason for this ambivalence of complement in physiological and pathophysiological processes is founded in its functional organization. In order to provide instant and effective protection against foreign intruders, complement invokes an elaborate network of soluble and cell surface-bound components, pattern-recognition proteins (PRP), proteases, receptors, effectors and regulators (Fig. 1) (Merle et al., 2015a; Ricklin et al., 2016). Under normal circumstances, specialized PRP detect danger-associated patterns on particle surfaces and initiate an enzymatic cascade that leads to the covalent attachment of opsonins (*i.e.* C4b and C3b proteins) via one of three principal initiation routes (termed classical, lectin and alternative pathway; CP, LP and AP, respectively). On the surface, C3b and C4b form enzyme complexes (termed convertases) that cleave the abundant plasma protein C3 into C3b than can again be deposited and form convertases, thereby fueling the rapid amplification of opsonization on unprotected surfaces. In many cases, this AP-mediated amplification acts as the major driving force and triage point of complement activation via any initiation pathway (Harboe et al., 2009; Harboe et al., 2004).

With progressing amplification, the convertases start cleaving the

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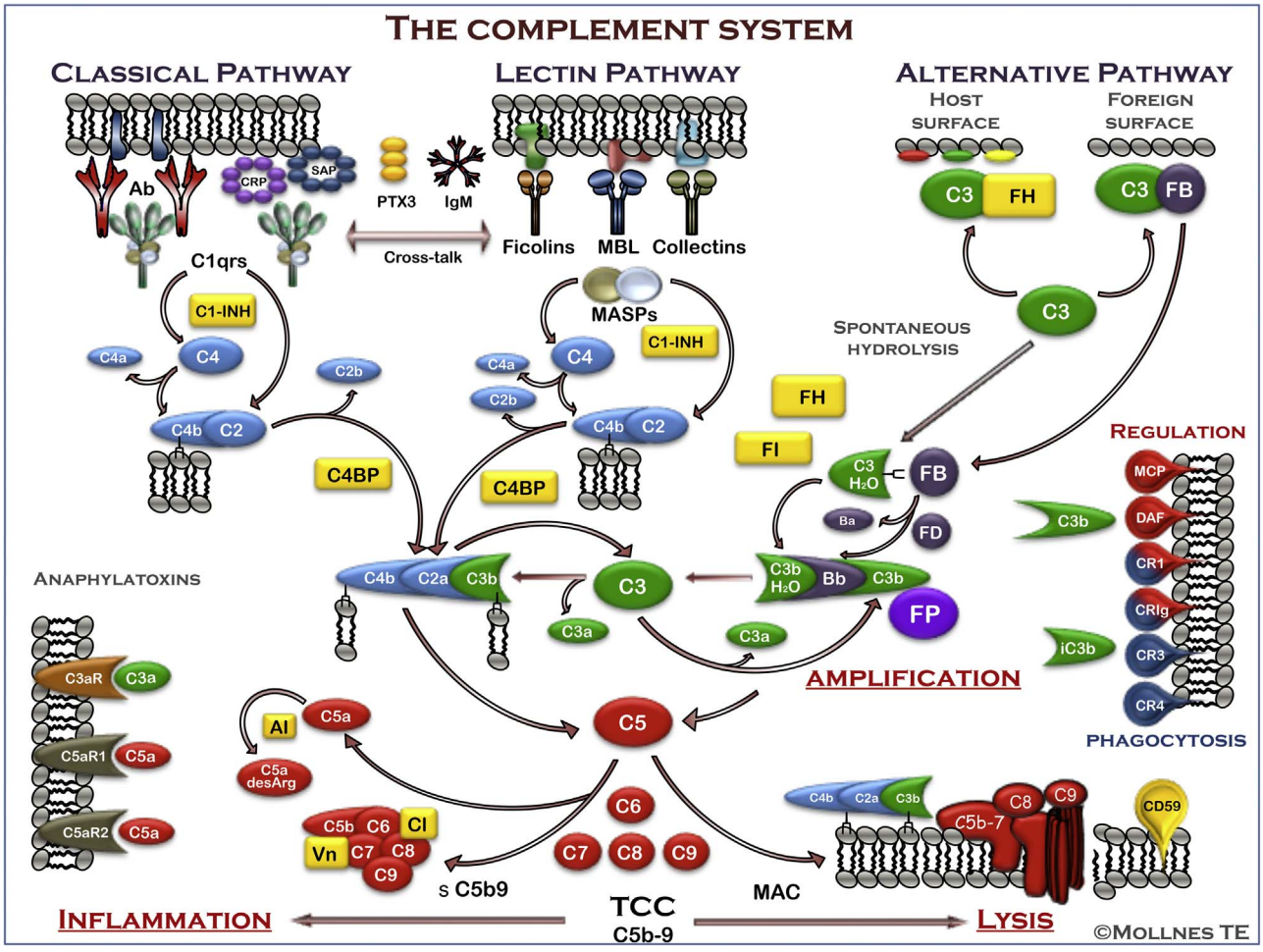

Fig. 1. *Schematic overview of complement activation on foreign particles or damaged host cell surfaces.* Binding of pattern recognition proteins (PRP) to danger markers initiates the cascade via the classical pathway (CP), where C1q recognizes antibodies but also mediators such as C-reactive protein (CRP) and serum amyloid P (SAP), or the lectin pathway (LP) with the main PRPs being MBL, ficolins and collectins. This leads to formation of CP/LP C3 convertases that cleave the abundant plasma protein C3 to C3a and C3b. Whereas C3a has immunomodulatory functions, the opsonin C3b can be covalently deposited on the triggering surface and form alternative pathway (AP) C3 convertases that cleave more C3 and, in absence of regulators, fuel an amplification loop that leads to rapid opsonization stabilized by properdin (FP), the only complement regulator with enhancing effects. The assembly of C5 convertases (e.g., C3bBb3bP) allows cleavage of C5 into the pro-inflammatory anaphylatoxin C5a and C5b. C5a is a highly potent proinflammatory mediator that activates C5a receptor (C5aR) 1, but also binds to the regulatory C5aR2, which might counteract the C5aR1 effects. C5b initiates the assembly of the terminal C5b-9 complex (TCC) which exists in two forms: the membrane attack complexes (MAC) when inserted into a membrane and the soluble sC5b-9 when formed in the fluid-phase. Whereas MAC may exert lysis of certain bacteria (e.g. *Neisseria)* or cell death (typically red cells), it also induces inflammation during sub-lytic cell activation. The opsonins C3b and its degradation products iC3b and C3dg bind to complement receptors (CR) on immune cells and facilitate adherence (C3b to CR1), phagocytosis (iC3b to CR3 and CR4), or mediate adaptive immune responses (iC3b and C3dg to CR2). Complement regulators are crucial to keep the system under control. Soluble regulators include C1-INH, C4b-bining protein (C4BP), Factor I (FI), Factor H (FH), anaphylatoxin inhibitors (AI, comprising carboxypeptidases), vitronectin (Vn) and clusterin (Cl). Membrane regulators include CR1, membrane cofactor protein, (MCP), and decay accelerating factor (DAF) at the level of C4 and C3, by destabilizing convertases and degrading opsonins, and CD59 at the level of C8 and C9 by preventing MAC formation.

C5 component, a fragment of which initiates the formation of lytic membrane attack complexes (MAC; C5b-9_n) that directly destroy or damage susceptible cells. The activation of C3 and C5 also liberates potent chemotactic fragments (*i.e.*, the anaphylatoxins C3a and C5a) that recruit immune cells to the site of activation and prime them. Professional phagocytes recognize opsonins on the attacked particles via complement receptors (CR), thereby mediating their phagocytic removal. Finally, complement effectors are shaping the downstream immune reaction by lowering the threshold of B cell activation and mediating T cell responses, among others (Merle et al., 2015b; Ricklin et al., 2016). In order to provide such broad and instant reactivity in case of threats, the complement system must not be cell-specific and opsonization may occasionally occur on host tissue. Our cells are generally protected from amplification and effector insult by a panel of complement regulators, which are expressed on their surface or recruited from circulation (Schmidt et al., 2016). However, if the balance between complement activation and regulation is out of tune, complement can quickly turn against the host and trigger and/or exacerbate adverse processes that result in diseases and clinical complications (Ricklin and Lambris 2013; Ricklin et al., 2016).

Whereas the pathophysiological involvement has long been recognized, it is only now that the extent of this 'dark side of complement' becomes apparent. Initial clinical awareness primarily focused on complement deficiencies, yet excessive activation and insufficient regulation are by far more common drivers of complement-mediated disorders. In principle, any foreign or altered/damaged host cell surface can trigger a complement response (Ricklin et al., 2016). The sudden exposure to a massive amount of danger pattern, as in the case of sepsis or trauma, can overwhelm the system and fuel a vicious cycle that causes tissue damage and systemic inflammation. Similarly, transplants or biomedical materials often trigger an adverse complement response that affect both the clinical and functional outcome of the treatment. And even though complement typically helps clearing cellular debris, the inability to do so efficiently can trap complement in an inflammatory state, as obvious in cases such as atherosclerosis or Alzheimer's disease. Any imbalance between activating and regulatory mechanism,