Immunobiology 221 (2016) 1247–1258

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Complement analysis 2016: Clinical indications, laboratory diagnostics and quality control

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ARTICLE INFO

Article history:
Received 1 May 2016
Received in revised form 10 June 2016
Accepted 10 June 2016
Available online 18 July 2016

Keywords:
Diagnostic techniques and procedures
Indication
Complement analysis
Pre-analytics
Quality control
External quality assessment (EQA)

ABSTRACT

In recent years, complement analysis of body fluids and biopsies, going far beyond C3 and C4, has significantly enhanced our understanding of the disease process. Such expanded complement analysis allows for a more precise differential diagnosis and for critical monitoring of complement-targeted therapy. These changes are a result of the growing understanding of the involvement of complement in a diverse set of disorders. To appreciate the importance of proper complement analysis, it is important to understand the role it plays in disease. Historically, it was the absence of complement as manifested in severe infection that was noted. Since then complement has been connected to a variety of inflammatory disorders, such as autoimmune diseases and hereditary angioedema. While the role of complement in the rejection of renal grafts has been known longer, the significant impact of complement. In certain nephropathies has now led to the reclassification of some rare kidney diseases and an increased role for complement analysis in diagnosis. Even more unexpected is that complement has also been implicated in neural, ophtalmological and dermatological disorders. With this level of involvement in some varied and impactful health issues proper complement testing is clearly important; however, analysis of the complement system varies widely among laboratories. Except for a few proteins, such as C3 and C4, there are neither well-characterized standard preparations nor calibrated assays available. This is especially true for the inter-laboratory variation of tests which assess classical, alternative, or lectin pathway function. In addition, there is a need for the standardization of the measurement of complement activation products that are so critical in determining whether clinically relevant complement activation has occurred *in vivo*. Finally, autoantibodies to complement proteins (e.g. anti-C1q), C3 and C4 convertases (C3 and C4 nephritic factor) or to regulatory proteins (e.g. anti-C1inhibitor, anti-factor H) are important in defining autoimmune processes and diseases based on complement dysregulation. To improve the quality of complement laboratory analysis a standardization commmittee of the International Complement Society (ICS) and the International Union of Immunological Societies (IUIS) was formed to provide guidelines for modern complement analysis and standards for the development of international testing programs.

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http://dx.doi.org/10.1016/j.imbio.2016.06.008
0171-2985/

1. Introduction

The complement system is a complex, evolutionarily well-conserved system. As a vital part of the body's innate immune system, complement provides a highly effective means for the elimination of 'waste' such as invading microorganisms, apoptotic and necrotic cells and immune complexes (Walport, 2001a,b). Furthermore, complement bridges the innate and adaptive immune response through modification of T- and B-cell responses by employing specific receptors on various immune cells (Carroll and Isenman, 2012). Today we know that complement also participates in hematopoiesis, reproduction, lipid metabolism and tissue remodeling (Ricklin et al., 2010).

Approximately 50 soluble and cell surface-attached proteins are currently recognized, including proteolytic components, cofactors, regulators and receptors of the entire complement cascade (Gros et al., 2008; Holers, 2014; Kemper et al., 2010; Ricklin et al., 2010). Complement genes are distributed across different chromosomes, with 19 genes comprising three significant complement gene clusters in the human genome (Mayilyan, 2012).

Complement can be activated via three major pathways, the classical, the alternative, and the lectin pathway, all of which merge in the activation of complement C3 and subsequently lead to the formation of the cytolytic membrane attack complex (MAC), C5b-9. However, complement activation may also occur by interaction of thrombin with complement C5, connecting complement to the coagulation system (Huber-Lang et al., 2002). Furthermore, properdin, known as the only positive regulator of the alternative pathway, has also been shown to specifically bind to pathogens and apoptotic cells, allowing the generation of C3 convertase on the target surface (Spitzer et al., 2007; Kemper et al., 2010) with subsequent opsonisation, i.e. covalent binding of C3b and iC3b. Following complement activation, the biologically active peptides C5a and C3a (anaphylatoxins) are released and elicit a number of proinflammatory effects, such as chemotactic recruitment of leukocytes, degranulation of phagocytic cells, mast cells and basophils, smooth muscle contraction and increase of vascular permeabiltity (Klos et al., 2009). Thereby, the inflammatory response is further amplified by subsequent generation of toxic oxygen radicals and the induction of synthesis and release of arachidonic acid metabolites and cytokines. Consequently, an (over-)activated complement system presents a considerable risk of harming the host by directly and indirectly mediating inflammatory tissue destruction (Ricklin and Lambris, 2013a).

Activation of complement is effectively controlled not only by limiting concentrations of proteins, such as factor D, and the rapid decay of the C3 and C5 convertases, but also by the coordinated action of soluble as well as membrane-associated regulatory proteins (Zipfel and Skerka, 2009). Soluble complement regulators, such as C1 inhibitor (C1-INH), C4b-binding protein (C4 bp), factors H (CFH) and I (CFI), clusterin and S-protein (vitronectin) control the action of complement in body fluids at multiple sites of the cascade. Equally important is the protection against complement attack on each tissue cell by membrane inhibitors, such as the complement receptor 1 (CR1/CD35), the membrane cofactor protein (MCP/CD46) as well as by the glycosylphosphatidylinositol (GPI)-anchored proteins, decay-accelerating factor (DAF/CD55), and CD59 (Zipfel and Skerka, 2009).

Modern diagnostic analysis now provides a comprehensive insight into the activation mode and state of the system allowing a better definition of disease genesis, severity, evolution and response to therapy (Mollnes et al., 2007; Tudoran and Kirschfink, 2012; Nilsson and Nilsson Ekdahl, 2012). With the introduction of complement targeted therapies such as eculizumab (Soliris ®) and more complement inhibitors in clinical studies (Ricklin and Lambris, 2013b; Morgan and Harris, 2015), high quality complement testing for primary diagnosis and for subsequent monitoring of the therapeutic outcome becomes indispensable. However, as is the case for all fields of immunodiagnostics the lack of standardization in complement analysis poses a major problem to disease recognition and follow-up.

Initiated by Prof. George Füst, Budapest, Hungary, in 2009, representatives of 18 international complement laboratories from 11 countries established the standardization committee of the International Complement Society (ICS) and the International Union of Immunological Societies (IUIS) to implement quality management in routine complement analysis. After preparation of standards for complement analytes the first external quality assessment began in 2010, now covering 39 complement diagnostic labs from 21 countries in 2015.

2. Indications for clinical complement analysis

Clinical and experimental evidence underlines the prominent role of complement in the pathogenesis of numerous inflammatory diseases including immune complex and autoimmune disorders (Figueroa and Densen, 1991; Botto et al., 2009; Ricklin and Lambris, 2013a). According to various national and international registries, complement deficiencies represent approximately 2–10% of all primary immunodeficiencies. In clinical practice, however, the consequences of an overactivated complement system with consumption of complement components as the cause of several inflammatory diseases and even life-threatening conditions are more apparent.

2.1. Clinical consequences of complement deficiencies

Genetic deficiencies of complement components are rare; their estimated prevalence is 0.03% (Figueroa and Denson, 1991; Grumach and Kirschfink, 2014). However, as an 'experiment of nature', these anomalies contribute significantly to our knowledge about the importance of this cascade system (Pettigrew et al., 2009; Skattum et al., 2011). Warning signs for complement deficiencies are (1) Meningococcal meningitis at > 5 years of age, (2) other recurrent bacterial infections, esp. with *Pneumococcus*, (3) autoimmune manifestations, (4) angioedema without urticaria, and (5) renal and ophthalmic inflammatory disorders (Grumach and Kirschfink, 2014). Defects of the early components of the classical pathway