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Protein ultrastructure and the nanoscience of complement activation ☆

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ABSTRACT

The complement system constitutes an important barrier to infection of the human body. Over more than four decades structural properties of the proteins of the complement system have been investigated with X-ray crystallography, electron microscopy, small-angle scattering, and atomic force microscopy. Here, we review the accumulated evidence that the nm-scaled dimensions and conformational changes of these proteins support functions of the complement system with regard to tissue distribution, molecular crowding effects, avidity binding, and conformational regulation of complement activation. In the targeting of complement activation to the surfaces of nanoparticulate material, such as engineered nanoparticles or fragments of the microbial cell wall, these processes play intimately together. This way the complement system is an excellent example where nanoscience may serve to unravel the molecular biology of the immune response.

Contents

Abbreviations: AFM, atomic force microscopy; C, component; CR, complement receptor; EM, electron microscopy; EOM, ensemble optimization method; Ig, immunoglobulin; MASP, MBL-associated serine protease; MBL, mannan-binding lectin; NMR, nuclear magnetic resonance; n.s., not stated; PGN, peptidoglycan; SANS, small-angle neutron scattering; SAS, small-angle scattering; SAXS, small-angle X-ray scattering; TED, thiol ester domain; XRC, X-ray crystallography.

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1. Introduction

The complement system forms a significant part of the humoral immune system. In a simplistic outline, the complement system acts to convert complement proteins from their soluble state into a surface-bound state, in this way labeling the surface as a target for several cellular components of the immune system. The ability of the complement system to support the uptake of microbial organisms through complement receptors (CRs) on leukocytes such as neutrophil granulocytes, monocytes, macrophages, and dendritic cells plays a critical role in keeping the body free of infections. However, these functions of complement also constitute a major barrier to pharmaceutical treatment involving particulate material, i.e., nanoparticles, which in many ways resemble natural targets for complement activation such as microbes or fragments of microbes. Insight into the basic science of the complement system is consequently contributing both to unravel functions of the immune system as well as developing new strategies for drug delivery.

The present review focuses on how nanoscience may help our understanding of the structural aspects of the molecular biology of the complement system. In Section 1 a basic outline of the role of the complement in the immune system is presented, followed by a discussion of the potentials of nanoscience in addressing the functions of the complement proteins. Section 2 reviews the methodologies for studying the structure of complement protein with emphasis on the work characterizing the ultrastructure at the nm scale. A central theme in nanoscience is the influence of size on the behavior of molecular system and Section 3 focuses on the evidence that the size of large complement proteins is a distinct feature in their function. Finally, in a synthesis of the topics presented in the preceding sections Section 4 describes how size, shape and surface topology play together in the complement-mediated response to microbial infections.

1.1. Overview of the role of the complement system in host immunity

The complement system was identified now more than 100 years ago as a heat-labile factor in blood serum that kills bacteria. With the advance of modern biochemistry and genetics the complement system is now known to comprise approximately 35 proteins, usually designated either as complement components (C) or complement factors. In addition several other proteins serve as part of the complement-activating complexes, notably the immunoglobulins IgG and IgM, or as CRs in the cell membrane of leukocytes.

The immune response from the complement system requires activation of the complement proteins. Several of the complement proteins are proteases. Activation of complement can for this reason be described as pertaining to the activation of these proteins from zymogens into enzymes. Currently, three basic modes of complement activation are referred to as the classical, lectin, and alternative pathways, respectively. It is the sequential order of activation of complement proteins, which have prompted the use of the "pathway" to name these processes. However, while the initiations of the pathways are biochemical distinct the downstream events involve shared components (Fig. 1). All three pathways converge in the activation of C3 by splitting this molecule into its fragments C3a and C3b, which exposes a reactive thiol ester permitting covalent attachment to targeted surfaces. Several excellent reviews have described these processes in details [1–3] and for this reason only a brief outline is given below.

Activation of the complement system through the classical pathway is mediated by IgG or IgM immunoglobulins synthesized by B lymphocytes. These molecules are often antibodies to certain epitopes in non-self molecules, e.g., molecules of microbial origin and complement activation are this way an important effector mechanism in the immunity to infection provided by antibodies. The activation is triggered by the binding of the C1 complex to antibodies through the recognition of the Fc part of the antibodies by C1q and the associated serine proteases C1r and C1s. Upon C1q binding of the antibodies C1r autoactivates and activates C1s, which then will cleave C4 and C2 permitting the deposition of C4 and C2 fragments on the surfaces. Following cleavage into C4a and C4b, C4b will expose a thiol ester that permits covalent bonding to surface-presented amine or hydroxyl groups. C4b forms a complex with C2, which is activated by C1s by cleavage into its fragments C2a and C2b. C2a cleaves C3 into its split products C3a and C3b, and the complex C4bC2a is consequently referred to as a C2 convertase. C4 and C3 are structurally similar proteins and the C3b fragment may form a covalent bond with the target surface in the same way as C4b. However, C3b may recruit activated Factor B (Bb) to the surface and form a C3 convertase (C3bBb), which also converts native C3 into C3a and C3b. This way a positive amplification loop is established, which is stopped by the conversion of C3b into iC3b by the Factors H and I [1–3].

Following the binding to surfaces presenting carbohydrate moieties containing carbohydrates such as D-mannose or *N*-acetyl-D-glusoamine, mannan-binding lectin (also known as mannose-binding lectin, abbreviated MBL or mannan- or mannose binding protein, abbreviated MBP) activates C4 and C2 through the MBL-associated protease (MASP)-2. While this points to a C1s-like property of MASP-2, evidence also suggests that MASP-2 is autocatalytic similar to C1r [4,5]. The role of MASP-1 is less clear. Some evidence seems to suggest that MASP-1 may directly activate C3 [6–8] or activate Factor D, which will then activate Factor B [9]. Other evidence suggests that MASP-1 serves to activate MASP-2 [10,11]. Importantly, the MASPs are found in complex with other proteins than MBL, namely the ficolins [12]. These proteins share some structural features with MBL [13]. However, they are not lectins, i.e., carbohydrate-binding proteins, but rather recognizing acetylated compounds, including acetylated carbohydrates [14,15]. With the addition of the ficolins as activators of the MASPs the lectin pathway is consequently covering more than modality of ligand recognition.

The alternative pathway of complement activation involves a spontaneous exposure of the thiol ester and surface deposition of C3, which is converted by Factor B into its surface-bound fragment C3b and the soluble C3a fragment. On host cells several molecular mechanisms will catalyze the breakdown of C3 convertases. If the surface presents no molecules that catalyze the degradation of C3b, as will be the case with many microbial surfaces, the positive amplification loop from the deposition of C3bBb convertases will lead to a massive activation of C3 [1–3].

Following the deposition of C3, the components C5–C9 may start to assemble a pore that, if inserted into a cell membrane, may cause lysis. For this reason the components C5–C9 are sometimes referred to as the lytic pathway. Unlike the classic, MBL, and alternative pathways, however, the lytic pathway has no independent means of activation [1–3].

The complement activation plays a major role in guiding the cellular components of the immune system. The split product C5a, from the cleavage of native C5, is a potent chemoattractant that will guide the migration of leukocytes to sites of complement activation. Deposition of C3b and its proteolytic cleavage products, notably iC3b, significantly enhances the ability of phagocytosing cell to take up bacteria or other microbes through complement receptors. Likewise, several studies have shown that B lymphocytes in addition to the recognition of antigenic substances by the B cell receptor require a second signal from CR2 binding of C3b to induce antibody formation [16–18]. The role of complement in stimulating T lymphocytes is less clear, but influences on T helper cell function have been reported [19,20].

1.2. Why is nanoscience a powerful way of studying the complement system?

The structural hierarchy of the human body ranges from features measured in meters to subnanometer scale of the atomic world. While