[S1-1]

A Novel Complement Fixation Pathway Initiated by SIGN-R1 Interacting with C1q in Innate Immunity

Young-Sun Kang

Department of Biomedical Science and Technology, Institute of Biomedical Science and Technology, Konkuk University, Seoul 143-701

Abstract

Serum complement proteins comprise an important system that is responsible for several innate and adaptive immune defence mechanisms. There were three well described pathways known to lead to the generation of a C3 convertase, which catalyses the proteolysis of complement component C3, and leads to the formation of C3 opsonins (C3b, iC3b and C3d) that fix to bacteria. A pivotal step in the complement pathway is the assembly of a C3 convertase, which digests the C3 complement component to form microbial-binding C3 fragments recognized by leukocytes.

The spleen clears microorganisms from the blood. Individuals lacking this organ are more susceptible to *Streptococcus pneumoniae*. Innate resistance to *S. pneumoniae* has previously been shown to involve complement components C3 and C4, however this resistance has only a partial requirement for mediators of these three pathways, such as immunoglobulin, factor B and mannose-binding lectin. Therefore it was likely that spleen and complement system provide resistance against blood-borne *S. pneumoniae* infection through unknown mechanism.

To better understand the mechanisms involved, we studied Specific intracellular adhesion molecule-grabbing nonintegrin (SIGN)-R1. SIGN-R1, is a C-type lectin that is expressed at high levels by spleen marginal-zone macrophages and lymph-node macrophages. SIGN-R1 has previously been shown to be the main receptor for bacterial dextrans, as well as for the capsular pneumococcal polysaccharide (CPS) of *S. pneumoniae*.

We examined the specific role of this receptor in the activation of complement. Using a monoclonal antibody that selectively downregulates SIGN-R1 expression *in vivo*, we show that in response to *S. pneumoniae* or CPS, SIGN-R1 mediates the immediate proteolysis of C3 and fixation of C3 opsonins to *S. pneumoniae* or to marginal-zone macrophages that had taken up CPS. These data indicate that SIGN-R1 is largely responsible for the rapid C3 convertase formation induced by *S. pneumoniae* in the

spleen of mice. Also, we found that SIGN-R1 directly binds C1q and that C3 fixation by SIGN-R1 requires C1q and C4 but not factor B or immunoglobulin. Traditionally C3 convertase can be formed by the classical C1q- and immunoglobulin-dependent pathway, the alternative factor-B-dependent pathway and the soluble mannose-binding lectin pathway. Furthermore Conditional SIGN-R1 knockout mice developed deficits in C3 catabolism when given *S. pneumoniae* or its capsular polysaccharide intravenously. There were marked reductions in proteolysis of serum C3, deposition of C3 on organisms within SIGN-R1^{+} spleen macrophages, and formation of C3 ligands. The transmembrane lectin SIGN-R1 therefore contributes to innate resistance by an unusual C3 activation pathway. We propose that in the SIGN-R1 mediated complement activation pathway, after binding to polysaccharide, SIGN-R1 captures C1q. SIGN-R1 can then, in association with several other complement proteins including C4, lead to the formation of a C3 convertase and fixation of C3.

Therefore, this new pathway for C3 fixation by SIGN-R1, which is unusual as it is a classical C1q-dependent pathway that does not require immuno globulin, contributes to innate immune resistance to certain encapsulated microorganisms.

Significance of this finding

The complement system is an integral component of innate immunity. Since the third pathway of complement activation, the mannan-binding lectin (MBL) pathway, was recently discovered, it has been thought that everything of interest had been already discovered in the field of complement system. However, we found the 4th 'An Unusual Complement Pathway' which is mediated by a transmembrane C-type lectin, SIGN-R1 (SIGN-R1 mediated complement activation pathway).

First, this finding provide an insight as to why the spleen is important for resistance to pneumococcal infection. In addition, these findings is the first example showing that a transmembrane C-type lectincan mediate C3 catabolism on its cellular surface. Furthermore, it is a critical finding that macrophages need complement system to efficiently protect the host against pathogens in innate immunity. Given cells of the immune system are equipped with many lectins and lectin-like receptors (LLRs, carbohydrate-binding proteins), functions of which still remain unknown in large part, these findings give a deep insight on unraveling functions of other lectins that could be intimately related with complement system. These findings also identify that a transmembrane C-type lectin could link between innate and adaptive immunity by mediating the localization of complement fragments into B cells or FDCs *in vivo*. Because the localization of antigens at B cells or FDCs should be considered important in an ongoing immune responses against pathogens, especially such as HIV or Prion, the further studies should be followed to examine the exact mechanism of the roles of transmembrane C-type lectins on the translocation of antigens

into B cells or FDCs. First of all, It will be most prior to all others to determin weather other transmembrane C-type lectins can mimic the SIGN-R1-mediated complement activation pathway, and these works could lead to unravel the potential therapeutic targets against pathogens.

Recent evidence suggests that complement and cellular-complement receptors also play important roles in the localization and retention of nonbacterial pathogens to FDCs, especially HIV-1 and PrPSc, a prion protein. Temporary depletion of C3 or genetic deficiency of C1q significantly delays the onset of scrapie following peripheral infection and reduces the early accumulation of PrPSc in the spleen, implying that in the early stages of infection, C3 and C1q contribute to the localization of scrapie infectivity in lymphoid tissue, i.e., a classical pathway for complement fixation. Interestingly, SIGN-R1 binds viral glycoproteins, as do human DC-SIGN and L-SIGN. While we have emphasized the role of SIGN-R1 during complement-mediated innate resistance to pneumococci, some viruses and prions may use this new splenic pathway for complement fixation.

Further studies

1. Study of The Role of Complement C3, Peroxiredoxin-1 and Bip/GRP78 in Regurgitation of Capsular Polyssacharide mediated by a C-type Lectin, SIGN-R1
2. Study of C-type lectin mediated Complement Activation Pathway (study of DC-SIGN mediated complement activation pathway.)
3. SIGN-R1 mediated Complement Activation Pathway and Autoimmune Disease.