

Compliments from complement: a fourth pathway of complement activation?

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Introduction

The immune system of vertebrates is known to be composed of innate- and acquired defense. While the innate immune system should potentially be sufficient for the defense against most pathogens, antibodies and activated T-lymphocytes strongly enhance our capacities for defense against a plethora of pathogens. The innate immune system is composed of a large number of defense molecules, including the complement system. The complement system is a major player in innate immunity and is strongly involved in a large number of biological processes, including the initiation and amplification of acquired immunity. Most of these biological activities are achieved by two mechanisms: first, the deposition of opsonic fragments especially of the components C3 and C4 on pathogens or other targets and second, the initiation or induction of inflammatory responses following release of small fragments e.g. from C3, such as C3a. Three pathways of complement activation exist, namely the classical pathway, the alternative pathway and the lectin pathway (Figure 1). They merge at the level of C3, leading to the activation of a common terminal sequence and generation of the C5b-C9 membrane attack complex that can insert itself into membranes and cause cytolysis. The lectin pathway of complement is activated following the recognition of specific carbohydrate patterns by molecules like mannan-binding lectin (MBL) and ficolins 1, 2 and 3 (Figure 2). Once, binding of these molecules to target structures has occurred, the MBL-associated serum protease-2 (MASP-2) is activated and then cleaves its natural substrates C4 and C2, leading to the formation of C4b2a, a C3-convertase that

cleaves C3 into C3b and C3a. C3b can attach itself in a covalent fashion to the activator and act as an opsonin.

Activation of C3 can also take place, independently of the lectin pathway, by the classical pathway which is known to be initiated e.g. by antigen antibody complexes, but also by dying cells and substances like DNA. Here, the recognition unit of the classical pathway, the complement component C1q, binds to specific sites on the activator, and then induces activation of the C1q-associated proenzymes C1s and C1r leading again to the activation of C4 and C2 and formation of the C3-convertase C4b2a. So, both activation of the lectin pathway and the classical pathway merge at the level of C4 and C2.

The third pathway of complement, the alternative pathway, is based on the continuous low-grade hydrolysis of C3 leading to the formation of an enzyme complex composed of C3 and activated B

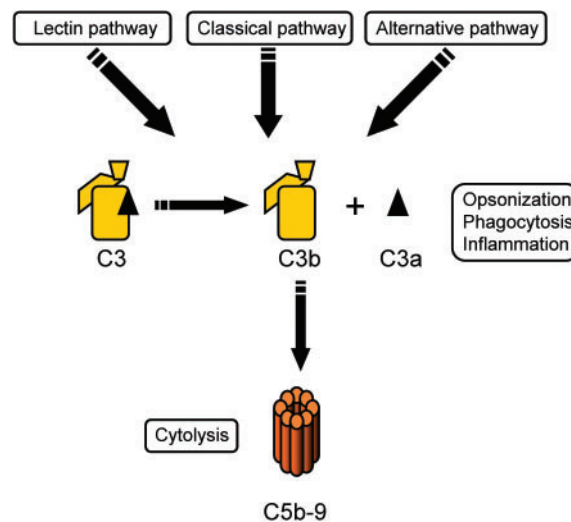


Fig. 1. Complement activation. Activation of C3, the central component of complement, may take place via three pathways leading to the generation of the opsonic fragment C3b. Once C3b is deposited on the activator in sufficient amounts, enhanced opsonization and phagocytosis may take place. Small fragments of C3, such as C3a may lead directly to enhanced local inflammation.

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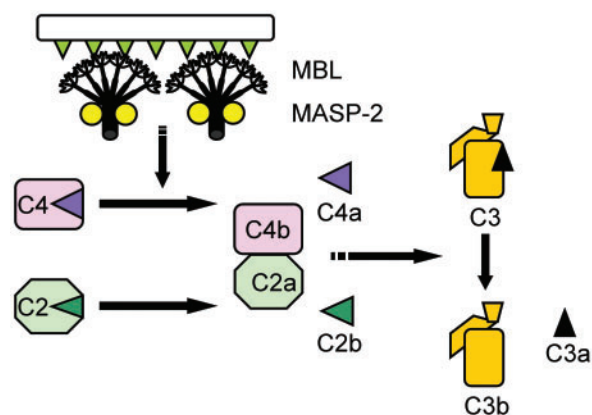


Fig. 2. Lectin pathway activation. The lectin pathway of complement is initiated e.g. following the binding of MBL to sugar moieties on pathogens or altered tissue, leading to activation of MBL-associated serum protease-2 (MASP-2). Activated MASP-2 can directly cleave C4 and C2, resulting in generation of the C3-convertase, C4b2a.

that then catalyses the activation of small amounts of C3 and generation of C3b. On alternative pathway activators, this C3b is protected from inactivation by the plasma inhibitors factor I and H. The initially formed C3b then reacts with factors B, D and properdin to form a stable amplification C3 convertase, leading to efficient C3 activation and the generation of opsonic and inflammatory fragments.

Based on the above-mentioned pathways of complement activation, one would not expect to see efficient C3 activation either by the classical or the lectin pathway in sera or individuals deficient in C4 or C2. However, already in the seventies it was observed that, when erythrocytes were loaded with high concentrations of IgG or IgM, traditionally known as classical pathway activators, cytolysis of these targets occurred, indicating a bypass of C4 and C2 in the activation of C3 [1].

In the study by Selander *et al.* [2], detailed molecular evidence is provided that bacterial antigens that bind MBL can also induce C3 activation in sera (made) deficient in C4, C2 and even MASP-2.

Discussion and implications

Using sensitive ELISA technology, the studies by Selander *et al.* [2] show that MBL can bind to serogroup O antigen-specific *Salmonella* oligosaccharides (CO). This binding can induce complement activation via the lectin pathway of complement, involving C4b2a. The primary novel finding of this study is that MBL can also directly support C3 activation and deposition on the activator in the absence of C2, C4 and even MASP-2, in sera with an intact alternative pathway function. The existence of an MBL-dependent C2 bypass mechanism for alternative pathway-mediated C3 activation was clearly demonstrated using different clinically relevant targets, i.e. CO, solid phase mannan and *Escherichia coli* lipopolysaccharide (LPS) (Figure 3).

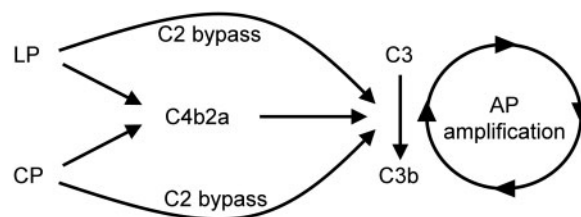


Fig. 3. C2 bypass pathway. Next to the regular pathways of C3 activation via the classical and lectin pathways, involving the C3 convertase C4b2a, a novel C2 bypass mechanism has been described recently [2] that involves MBL but not C4, C2 or MASP-2. Similarly, a C2 bypass mechanism has been described for the classical pathway.

These findings potentially suggest that C3b deposited on these activators in the presence of MBL may be protected from inactivation by factors I and H and thereby support amplification of C3 cleavage by the alternative pathway. Alternatively, it cannot be excluded that other proteases, including MASP-1, are involved. In molecular terms, further analysis of the detailed mechanisms involved in this process is required. The identification of the exact mechanism of initial C3 cleavage via the C2 bypass pathway would be a major step forward.

Are there any examples for a C4/C2 bypass in the clinical situation?

Following the work on a C2 bypass via the classical pathway, mainly based on studies using sera of guinea pigs deficient in C4 and C2, further studies with serum of a patient with systemic lupus erythematosus and a complete C2 deficiency revealed a total haemolytic complement titre of about 50% of normal [3]. However, additional analysis revealed that this assay system was dependent on the formation of an alternative pathway C3 convertase, C3bBb, independent of the classical pathway.

The results presented by Selander *et al.* [2] now demonstrate *in vitro* MBL-mediated C3b opsonization of pathogens in patients sera deficient for C4 and C2. These results imply that MBL may also support innate defense in C4- or C2-deficient patients. In view of the high functional heterogeneity of MBL function in the human population, it might be worthwhile investigating whether C4- or C2-deficient patients benefit from their MBL function. Such results would greatly support the clinical relevance of the MBL/C2 bypass pathway.

In IgA nephropathy, it has been previously documented that deposition of IgA is associated with alternative pathway activation, as concluded from the co-deposition of properdin in renal biopsies together with C3 [4]. Indeed, *in vitro* studies have provided evidence that polymeric IgA can efficiently activate the alternative pathway. More recent studies have shown that pIgA can also activate the lectin pathway of complement and that this occurs by the binding of MBL and subsequent activation of C4, and C3 [5].

Further studies have revealed that patients with IgAN who exhibit MBL deposition in their kidneys have a more progressive decline of renal function [6].

It is possible that in this group of patients, both lectin pathway activation and alternative pathway activation takes place and, although a co-deposition of MBL and C4d was observed, it cannot be excluded that a C4/C2 bypass mechanism may also be involved. It is expected that further studies in patients and in experimental models will provide a better insight into the C4/C2 bypass mechanisms in the near future.

Conflict of interest statement. None declared.

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