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The phylogeny of the complement system and the origins of the classical pathway

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Abstract

The origins of the complement system have now been traced to near to the beginnings of multi-cellular animal life. Most of the evidence points to the earliest activation mechanism having been more similar to the lectin pathway than to the alternative pathway. C1q, the immunoglobulin recognition molecule of the classical pathway of the vertebrates, has now been shown to predate the development of antibody as it has been found in the lamprey, a jawless fish that lacks an acquired immune system. In this species, C1q acts as a lectin that binds MASPs and activates the C3/C4-like thioester protein of the lamprey complement system. The classical pathway can, therefore, be regarded as a specialised arm of the lectin pathway in which the specificity of C1q for carbohydrate has been recruited to recognise the Fc region of immunoglobulin.

Keywords: Complement; Classical pathway; Evolution

Introduction

The phylogeny and evolution of complement has been of interest to researchers for almost as long as the system has been known. As early as 1903, Flexner and Noguchi (1903) studied the activation of complement from a number of species by a range of snake venoms, probably via the alternative pathway. A year later, Marshall investigated the lysis of erythrocytes from different species by a range of animal sera (Marshall, 1904). No real patterns emerged from these studies and the potency of the sera showed little correlation to the degree of relatedness of the species studied, or to their kinship to the target cells. By the early 1970s, most studies of complement phylogeny were still centred on the haemolytic ability of various sera and the compatibility between components from different species in assays (Gigli and Austen, 1971). In the 1980s, there was an explosion of data at the protein level on complement components from vertebrate species in all classes (Dodds and Day, 1993). The 1990s saw an equally dramatic increase in our knowledge, mainly through nucleotide sequencing following PCR based on conserved sequences. The age of genomics has brought forth unexpected information on a range of vertebrates and invertebrates (Nonaka and Kimura, 2006). In many cases, there is little or no functional evidence to link the presence of complement component-like nucleotide sequences with complement-like activity. There is,

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however, good evidence that the arthropod, horseshoe crab, has both thioester containing proteins and amplification enzymes, related to C3/C4 and C2/Factor B, that function in a complement-like opsonic system (Zhu et al., 2005). The coral *Swiftia excerta* has a thioester containing protein that is more similar to the vertebrate complement components C3 and C4 than it is to either α2macroglobulin or the thioester proteins (TEPs) found in insects (Dishaw et al., 2005). There is no functional data on this protein, but if it should turn out that corals/jellyfish have complement-related systems, then this dates the system's origin at near to the emergence of multi-cellular organisms.

Fig. 1 summarises what is known of complement-related systems in all of the animal groups tested to date. Exact definition of complement-related function is problematic. The shading indicates that there is evidence for activities within an animal group that resemble complement mediated activities that have been well characterised in mammals. It would, however, be unwise to assume that complement-like opsonisation in arthropods is identical in molecular terms to that seen in mammals. Similarly, the activation pathways have been clearly defined, only in mammals (really only in humans). In Fig. 1 and throughout this article, we have used very loose definitions.

Classical pathway

As described in most textbooks, this pathway is activated predominantly by antibody via C1q binding, and hence is present only in species with immunoglobulins. Quite probably at some stage in the evolution of the classical pathway, C1q recognised carbohydrate structures on immunoglobulins; it can therefore be regarded as a variant of the lectin pathway. This definition leads one to regard activation by direct binding of C1q to pathogen-associated molecular patterns (PAMPs), DNA, etc. as happens in higher vertebrates as well as in lower vertebrates that lack antibodies as more lectin pathway-like.

Lectin pathway

This is activated by recognition molecules through mechanisms similar to those of the classical pathway. MBL, ficolin and lower vertebrate C1q have been identified as initiators but other PAMP recognition molecules could be present in some species. The initiating enzyme (MASP is well characterised but again others are possible) is immobilised by attachment to the recognition molecule and circulates as a zymogen. If an amplification enzyme is present it must be activated by the MASP immobilised on the recognition molecule, thus limiting the deposition of TEP to a very short distance from the recognition molecule. The amplification enzyme is therefore more C2-like than factor B-like.

Alternative pathway

As described in textbooks, there is no recognition molecule, everything, including self is attacked at a very low level but self is protected from amplification of the response by multiple soluble and membrane-bound inhibitors. The initiating enzyme (factor D is well characterised but other enzymes could fill this role) is present in the fluid phase as an active enzyme rather than as a zymogen. As the initiating enzyme is not attached to a recognition molecule deposition of TEP, it can spread very rapidly and distantly from the initial site of activation.

There is a lot of crossover between the pathways which makes precise definitions impossible, for example, antibody antigen aggregates can activate the alternative pathway, without the participation of C1q. Antibody can also activate the lectin pathway via MBL recognition of the carbohydrate on immunoglobulin. C1q can bind directly to certain PAMPs, activating the complement in the absence of antibody. These activities have been described mainly by careful dissection of the human system. The functional assays used by investigators of diverse species are often incapable of differentiating between them.

In higher vertebrates, activation of the classical or lectin pathways causes the deposition of C3b onto surfaces; this leads to the activation of the alterative pathway amplification mechanism in which factor B binds to the C3b and is activated by factor D, the C3bBb can then activate more C3b distant from the initial recognition site. To date, this amplification mechanism has been demonstrated only in vertebrates where multiple control proteins are present to prevent damage to self.

The definition of a complement component is also difficult. It is perhaps dangerous to ascribe function based solely on similarity in the primary structure of a new protein to a mammalian complement component. However, most of the data that is available is of this type. In Fig. 1, an *x* indicates that either a protein has been isolated and partially characterised or that a cDNA or genomic DNA sequence is available. Those occasions where corroborating functional evidence is available are discussed in the text. Shading alone indicates that phylogenetic considerations suggest that a component should be present. However, as will be discussed later, this assumption may not always be true. For example, α2macroglobulin and a C3/C4-like TEP are found in one arthropod, the horseshoe crab, but appear to be absent in two other arthropods, drosophila