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Review

Looking back on the alternative complement pathway

Peter J. Lachmann

Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, CB3 0ES, United Kingdom

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ABSTRACT

The alternative pathway of complement originated from the Properdin pathway originally described by the Pillemer laboratory in the 1950s. This work generated great controversy and it took several decades for a consensus on its components, its reaction sequence and its functions to emerge. This paper reviews this history and attempts to clarify some of the ambiguities that remain.

1. Introduction

It is not the intention of this paper to review in great detail all that is known of the "alternative complement pathway". There have been several reviews in recent years (Bexborn et al., 2008; Lachmann, 2009; Nilsson and Nilsson Ekdahl, 2012; Harrison, 2018) the last of which in particular goes into the subject in great detail. However, there does appear to remain a degree of misunderstanding of some aspects of this pathway particularly in the role of the "tickover" which is still frequently shown in diagrams of the alternative pathway as its activating event – which, as is discussed below, it is not. The intention of this paper is to look again at what the alternative pathway is and what it does and to suggest that it should be regarded in a different light from the classical and lectin pathways, serving in large part as an amplification mechanism for all ways of activating the complement system – a conclusion similarly reached by Harrison (2018).

2. History

What became known as the alternative complement pathway originated with the work of the Pillemer laboratory when the first paper on "the properdin system and immunity" was published by Pillemer et al. (1954) . This work gave rise to a series of some fourteen papers followed by immense controversy which are comprehensively reviewed by Lepow (1980). It is perhaps just worthwhile with hindsight to look at this early work in outline again. The Pillemer group used zymosan, a preparation of yeast cell walls that is pure carbohydrate, as a complement activator. It was already then known that treating human serum at 37 °C with zymosan depleted it of what was then called "C'3", which then described all the components needed after C1, 4 and 2 to produce lysis. Treating guinea pig serum with zymosan does remove all C3 (in its contemporary sense) so that the resulting reagent, known as "R3", can be used to generate EAC142 when added, in the presence of calcium, to antibody-coated erythrocytes. This is not the case with human serum where the R3 reagent seems to exhaust Factor B rather than C3 and the human "R3" does contain appreciable amounts of C3. When used to treat antibody-coated erythrocytes in the presence of calcium and magnesium human R3 gives rise to the intermediate EAC1423bi. None of this was known in the 1950s since C3, the first complement to be isolated as a protein, was not described until 1960 (Müller-Eberhard and Nilsson, 1960).

The observation that formed the basis of the work on properdin was that when zymosan was incubated with human serum at 17 °C, it removed a component (to which the name properdin was given) which was necessary for zymosan to produce an R3 reagent at 37 °C. Properdin was subsequently purified by Pensky et al. in 1968 by which time it had also been discovered that there were other components required for the fixation of properdin to zymosan to take place. These were recognised as being similar to the classical complement components C1, C4 and C2. Looking back at these experiments with the hindsight of over sixty years, it does seem to be the case that at 17 °C there is a reaction that allows covalent binding of C3b to zymosan where it may bind some Factor B (the C2-like component of the properdin pathway), and that this then allows the binding of properdin to C3b or C3b(B). The exact nature of the reaction that allows the C3 fixation is still not wholly clear. Pillemer and his group used a resin to deplete cations and showed that they needed to restore only magnesium, rather than calcium, in order for this reaction to work. If this resin removed all calcium, then one can exclude the classical pathway as being involved. Otherwise it certainly would be activated, since human serum contains antibodies to the many carbohydrate determinants that are found on zymosan. Total absence of calcium would also exclude involvement of much of the lectin pathway (which was quite unknown at that time) since the C-type lectins – mannose binding lectin, and the collectins – also require calcium for their activity. On the other hand, it is now known that ficolins are another group of proteins that can activate the lectin pathway.

E-mail address: pjl1000@cam.ac.uk.

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These are not C-type lectins but use fibrinogen-type recognition domains which, generally, do not require calcium for binding their ligands (Garlatti et al., 2010). It is therefore quite plausible that this reaction at 17 °C which allows the covalent fixation of C3 onto zymosan is mediated by the lectin pathway using ficolins. Furthermore, the discovery by Yaseen et al. (2017) that MASP-2, the principal enzyme involved in lectin pathway activity going via C4 and C2, can itself produce sufficient cleavage of C3 to activate the alternative pathway, provided another route by which lectin pathway activation can interact directly with the alternative pathway. It is quite likely, therefore, that what Pillemer was observing was due, in part at least, to lectin pathway activation. It is also possible that it is the "protected surface" property of zymosan – its capacity of allowing bound C3b to bind Factor B in preference to Factor H − that allows some C3 fixation to occur. However, the C3b amplification loop does not work well at 17 °C and in order to get appreciable C3 fixation by such a mechanism one would anticipate the need for a much higher temperature.

What generated controversy was, largely, the claims that properdin was a major player in innate immunity to viruses, bacteria and tumours. There is some dispute whether Pillemer made these claims in quite the way it was reported in the popular press at the time (see the discussion by Colten quoted in Lachmann (2006) but these claims certainly raised the profile of the properdin system and also, probably, encouraged the scepticism. The major sceptic was Robert Nelson who in 1958 published "an alternative mechanism for the properdin system" having previously presented his criticisms at meetings. Nelson's view was that basically the properdin experiments were simply demonstrating the activity of the classical pathway that involved antibodies and classical pathway components. His experiments were, however, done in large part on guinea pig serum which, as already mentioned, is not an entirely good analogue for human serum with regard to reactions with zymosan and certainly what Nelson described would have required the presence of calcium. In retrospect, therefore, his criticisms do not look as potent as they were considered at the time.

Properdin was shown to act as a stabiliser of the alternative pathway convertase C3bBb (Fearon and Austen, 1975) and as such has an important physiological role. Properdin deficiency is associated with meningococcal infections, as are so many other complement deficiencies. Moreover, it is now established that properdin, as it occurs in plasma, reacts only with C3b (Harboe et al., 2017) in spite of more recent claims (Hourcade, 2006; Kemper et al., 2010) that properdin may act as a recognition molecule and therefore could act as an initiator of the complement alternative pathway along the lines originally postulated by Pillemer. However, larger polymers of properdin do have some capacity of this type. These polymers can be found in properdin purified from plasma where they are an artefact, as was first shown by Farries et al. (1987). However, it has more recently been shown that properdin made by recombinant techniques (Ali et al., 2014) has similar properties to the artefactual properdin polymers and this recombinant properdin has been shown to be a powerful stimulant of complement activity by stabilising the C3 convertase. Whether properdin found at extravascular sites in vivo, possibly made by polymorphs, may contain or comprise these larger polymers is unknown and it is therefore still unclear whether these larger properdin polymers can occur *in vivo*.

3. Evolutionary history

In contrast to its discovery history, in evolutionarily terms the alternative pathway can be considered to form the oldest part of the complement system (see Lachmann, 1979 and Lachmann, 2009 for a more detailed account). A C3 like molecule can be found in insects and a Factor B-like protein in echinoderms. In these invertebrates the invading micro-organisms presumably supplied the enzymes needed to cleave C3 and Factor B. In vertebrates with a pumped circulation which contains a wide variety of protease inhibitors the remainder of the alternative pathway evolved (see Figure). Interestingly, echinoderms were also shown to be able to activate the C3 like molecule through analogues of lectin pathway components (Fujita, 2002) indicating that the lectin pathway by far antedates the classical pathway activation route. The appearance of the latter somewhat paralleled the evolutionary rise of antibodies, the most potent inducers of classical pathway activation.

The classical pathway seems to have developed in vertebrates largely by gene duplication to provide a feed-in to the alternative pathway particularly from the humoral adaptive immune system; and the lectin pathway to allow activation of the classical pathway using lectins recognising pathogen associated carbohydrate determinants and thus providing a further feed-in from the innate immune system.

4. The renaissance of the alternative pathway

The renaissance of the alternative pathway following the controversies of the 1950s and early '60 s came from a quite different direction which was the demonstration that there were various ways of activating complement that did not involve the early classical pathway components. Gewurz et al. (1968) showed that lipopolysaccharides could consume what had been known as C'3 - which by that time was known to be all the components from C3 to C9 - without consuming C1 and C4. It was shown that precipitates made with guinea pig IgG1 antibodies (Sandberg et al., 1970) or with rabbit Fab'2 (Reid, 1971) had similar properties. In 1971, Frank et al. showed that C4-deficient guinea pig complement was able to be activated using suitable activators. These findings made it amply clear that there was a mechanism of activating the complement system that did not require C1, C4 and C2 and this renewed the study of what other components were involved in this pathway. It had already been found that these showed analogy to the classical pathway components. There was a C2-like (heat labile) protein which was demonstrated as a novel protein by Boenisch and Alper, 1970 and who named it glycine-rich beta glycoprotein (GBG). A similar protein was described by Götze and Müller-Eberhard in 1971 and was called by them C3 proactivator and again by Goodkofsky and Lepow in 1972, from what had been the Pillemer laboratory, who called it Factor B by analogy with the original Pillemer nomenclature and this name was finally adopted. There was also a C4-like (hydrazine sensitive) protein which was known as properdin Factor A and this was demonstrated by Müller-Eberhard and Götze in 1972 to be C3 itself. A C1-like component was isolated by Alper and Rosen (1971) who called it glycine-rich beta glycoproteinase (GBGase) and the following year by Müller-Eberhard and Götze (1972) who called it C3PA convertase. This protein was subsequently called Factor D.

In the early 1970s these laboratories were of the opinion that this alternative pathway would have an initiation rather like the classical pathway and it was speculated that this would start, by analogy with the classical pathway, with the activation of Factor D from a precursor form (Fearon et al., 1979). The Müller-Eberhard laboratory (Vallota et al., 1974; Schreiber et al., 1976a, 1976b) believed that the initiation event involved a novel initiating factor which was a naturally occurring analogue of C3 nephritic factors, which had first been described by Spitzer et al. (1969). However, neither of these hypotheses turned out to be correct. There is a zymogen form of Factor D but normal serum always contains active Factor D and the activation of the zymogen probably occurs largely extra-vascularly near the site of synthesis of Factor D, which is largely by adipocytes. It has recently been demonstrated that the enzyme that is largely concerned in this activation is MASP-3 (Dobó et al., 2016), one of the enzymes of the lectin pathway, and the only one that is resistant to inhibition by C1 inhibitor (Zundel et al., 2004) and therefore has a prolonged active half-life *in vivo*. The initiating factor turned out not to exist at all. Nephritic factors, contrary to the vigorous claims of the "La Jolla group" (i.e. Müller-Eberhard and co-workers), are indeed immunoglobulins and they are a curious set of autoantibodies to the alternative pathway C3 convertase which cause