Cholesterol-Dependent Cytolysins

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Abstract

The cholesterol-dependent cytolysins (CDCs) are part of a large family of pore-forming proteins that include the human proteins perforin and the complement membrane attack complex. The activity of all family members is focused on membranes, but the proteins are themselves involved in a diverse range of phenomena. An overview of some of these phenomena is provided here, along with an historical perspective of CDCs themselves and how our understanding of their mechanism of action has developed over the years. The way in which pore formation depends on specific characteristics of the membrane under attack as well as of the protein doing the attacking is emphasised.

The cholesterol-dependent cytolysins (CDCs) have been the focus of a renewed keen research interest for over ten years now.1-4 Their importance has been even further enhanced by the homology now identified between them and the membrane attack complex/perforin (MACPF) family of proteins, which includes several components of the complement cascade as well as perforin itself.5-9 In this chapter I aim to provide an overview of our understanding of the interaction between CDCs and other members of what is now called the MACPF/CDC superfamily, with their target membranes.

CDCs (also in the past known as thiol-activated toxins or cholesterol-binding toxins) were originally identified from four Gram-positive bacterial genera (Clostridium, Listeria, Bacillus and Streptococcus). Well-known examples include listeriolysin, perfringolysin, streptolysin and pneumolysin. Listeriolysin from L. monocytogenes is responsible for the escape of bacteria from the phagosome to colonise the cytoplasm10 and has been applied as a protein adjuvant in the development of vaccines against cancer and tuberculosis, for example.11-13 Perfringolysin from C. perfringens (Fig. 1A) has become perhaps the most studied CDC4 and has an important role in pathology associated with infection (gangrene).14-16 Streptolysin from S. pyogenes is another intensely studied CDC and has been applied widely in experimental permeabilisation of biological membranes.17,18 Pneumolysin is a major virulence determinant for S. pneumoniae, allowing bacterial invasion of tissues and mediating inflammation and the activation of the complement cascade.19,20 However, CDCs have now, for example, been identified in the bacteria Arcanobacterium pyogenes and Gardnerella vaginalis8 and there also appear to be homologues outside prokaryotes such as the sea anemone Metridium senile pore-forming toxin metridiolysin.21 The homology with the MACPF family was unknown until the first structures of the canonical fold of that family were solved, revealing the now characteristic MACPF/CDC fold of a twisted β-sheet around which helices are clustered (Fig. 1A and D). Without any significant other sequence homology, the fold of this superfamily of pore-forming and membrane-binding proteins has been conserved by compensatory mutation around a handful of key conserved glycines.6,8,9 The glycines presumably act as critical hinges during the dramatic refolding that CDCs are known to undergo and which
is presumably the selective advantage of this specific structure that has caused it to be conserved over such a vast evolutionary timescale. While not all MACPF domains are involved in pore formation—for example, C6 and C8β—they are all apparently involved in action on membranes.8,22

The dramatic refolding undergone by CDCs is tightly coupled to their oligomerisation and results in the conversion of the helices hemming the core β-sheet of the MACPF/CDC domain into a pair of β-hairpins which in tandem and alongside those from other subunits within the oligomer insert into the membrane to create a pore2-4,23-27 (Fig. 1A-C). It is obviously the basic assumption that where nonCDC members of the superfamily—such as complement proteins and perforin—act on membranes they do so by a mechanism involving similar refolding.23 Even where a member of the MACPF/CDC superfamily is not known to form a pore, or has been shown not to—at least alone—the same conformational change could have other adaptive functions during activity on or at membranes. However, the bicomponent nature of some pore-forming toxins28 alerts us that showing an absence of activity for one pure protein does not mean that they do not contribute to pore formation quite directly, since that may require the presence of another MACPF/CDC family member or members from the same specific system. Complement acts by a combination of the C5b-8 complex of proteins preassembled on a target membrane recruiting C9 to form a lesion, which may be a complete ring of C9 associated with the C5b-8 or an arc—electron microscopy