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Review

Perforin-2/Mpeg1 and other pore-forming proteins throughout evolution

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ABSTRACT

Development of the ancient innate immune system required not only a mechanism to recognize foreign organisms from self but also to destroy them. Pore-forming proteins containing the membrane attack complex Perforin domain were one of the first triumphs of an innate immune system needing to eliminate microbes and virally infected cells. Membrane attack complex of complement and Perforin domain proteins is unique from other immune effector molecules in that the mechanism of attack is strictly physical and unspecific. The large water-filled holes created by membrane attack complex of complement and Perforin domain pore formation allow access for additional effectors to complete the destruction of the foreign organism via chemical or enzymatic attack. Perforin-2/macrophage-expressed protein 1 is one of the oldest membrane attack complexes of complement and Perforin domain protein involved in immune defense, and it is still functional today in vertebrates. Here, we trace the impact of Perforin-2/macrophage-expressed protein 1 from the earliest multicellular organisms to modern vertebrates, as well as review the development of other membrane attack complexes of complement and Perforin domain member proteins. *J. Leukoc. Biol.* 98: 761–768; 2015.

Introduction

As multicellular organisms evolved, a system of protection was necessary to ward off foreign organisms that were detrimental to the host's survival. This need leads to the evolution of first, the innate and later, adaptive immune systems. The innate immune system is an ancient, protective mechanism that likely exists in some capacity for all multicellular organisms. In contrast, the adaptive immune system evolved ~400 million yrs ago with the evolution of vertebrates. Both the adaptive and innate immune systems work together to detect and eliminate foreign organisms.

The innate immune system acts as a first line of defense, primarily through relying on a series of genetically encoded receptors to detect foreign organisms. These receptors were named PRRs [1], and PRRs that have evolved to detect conserved microbial structures, often characteristic of a major pathogen class, are known as PAMPs [2, 3]. As a result of the germ line-encoded recognition of these receptors, the number of targets that are detectable is finite, corresponding with the total number of PRRs present in the organism.

In contrast to the innate immune system's targeting of pathogens through germ line-encoded microbial patterns, the adaptive immune system relies on gene recombination and somatic gene mutation to generate a massive library of antigen receptors with random specificities. Whereas this recognition process does lead to the development of a nearly unlimited antigen recognition library, this process does have the caveat of being much slower to deploy than the innate immune system's genetically preloaded recognition library.

Over the course of the immune system's evolution, many techniques were used to remove foreign organisms that were detrimental to the host's survival. Examples of these strategies range from release of well-characterized antimicrobial compounds, such as lysozyme and reactive oxygen species, to opsonization and neutralization by antibodies.

One of the earliest effector molecules in the immune system is the class of pore-forming proteins. Like the innate immune system's PRRs, nearly all multicellular organisms have the ability to deploy pore-forming proteins. The MACPF domain has demonstrated its importance to the host's survival, given the robust maintenance and expansion of pore-forming proteins containing the MACPF domain throughout evolution. MACPF domain pore formation is unique, in that after release, its mechanism of attack is purely a physical process that is also unspecific. MACPF proteins are generally synthesized as individual components that then assemble into a membrane-inserted pore on the foreign invader through MACPF domain polymerization [4–11]. MACPF-generated pores act to disrupt the barrier

Abbreviations: CDC = cholesterol-dependent cytolysin MAC = membrane attack complex of complement [terminal subunits of complement made up of C5b-C6-C7-C8-C9(n)], MACPF = membrane attack complex of complement and Perforin domain, Mpeg1 = macrophage-expressed protein 1 (hereafter, referred to as Perforin-2; PAMP = pathogen-associated molecular pattern, Pfpl/EPCS50 = pore-forming, protein-like, present within mouse-invasive trophoblast giant cells PRR = pattern recognition receptor siRNA = small interfering RNA

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function of target membranes and also provide access for chemical or enzymatic effectors that finalize destruction of the target [11–15].

PERFORIN-2/MPEG1 IN INVERTEBRATES

Sponges represent the oldest extant metazoan phylum, yet these animals possess effective innate defense systems against microbes and parasites [16]. Sponges are likely exposed to a greater amount of foreign viruses and unicellular or multicellular organisms than most vertebrates, in part, as a result of the huge amount of water filtered to extract edible material (0.002–0.84 cm^3 of water/s per cm^3 of sponge tissue), coupled with an environment that can be plentiful in bacteria and viruses ($>10^9$ inclusion-forming units/ml water) [17, 18]. Thus, this combination of the abundance of microorganisms in the water and the amount of filtered water results in a significant burden of foreign organisms that sponges need to eliminate.

Müller and colleagues [19, 20] have demonstrated that sponge cells respond to both Gram-positive and -negative bacteria through increased synthesis of lysozyme, endocytic activity, and a pore-forming execution protein in SDMPEG (homologous with the mammalian pore-forming protein Perforin-2/Mpeg1 and hereafter, named Perforin-2). Perforin-2 is significantly up-regulated transcriptionally and translationally from an undetectable basal level in the outer cell layers of the sponge tissue after exposure to LPS. The presence of the MACPF domain in combination with the increase in protein expression following exposure to bacterial PAMPs is suggestive of potential antibacterial activity of this ancient member of the Perforin-2 family [20]. A proposed schematic of the different domains of Perforin-2, as well as 2 potential orientations with the effector MACPF domain, is shown in **Fig. 1**.

The up-regulation of Perforin-2 expression following bacterial challenge is not exclusive to porifera. The increase of Perforin-2 at the transcriptional and translational level following bacterial challenge has been demonstrated with numerous mollusks and echinoderms [21–26]. Perforin-2 is particularly well defined in abalone (gastropod mollusks). Unlike sponges, the gastropod's immune system consists of both cellular and humoral defense systems. The cellular defense system of abalone is primarily driven by hemocytes, which recognize pathogens from PRR motifs and eliminate pathogens by phagocytosis. The humoral arm of immune defense in abalone is driven by hemolymph-containing opsonizing factors, lysozyme, and soluble antimicrobial factors [27, 28].

Following bacterial infection in abalone, the circulating hemocytes demonstrate no up-regulation of Perforin-2 message in the first 24 h but a significant and progressive up-regulation between 48 and 96 h postinfection relative to mock [21]. In comparison, when tissues are sampled from the epipodia (lateral ridges of the foot) following bacterial infection, a significant transcriptional up-regulation is observed within the first 24 h and remains elevated through 96 h postinfection [21]. Perforin-2 up-regulation following bacterial exposure was also observed in oysters, where organs exposed to the environment (gills and digestive glands) expressed a higher basal Perforin-2 message than other tissues. Similar to abalone, the hemocytes up-regulated Perforin-2 message several hours postinfection [24].

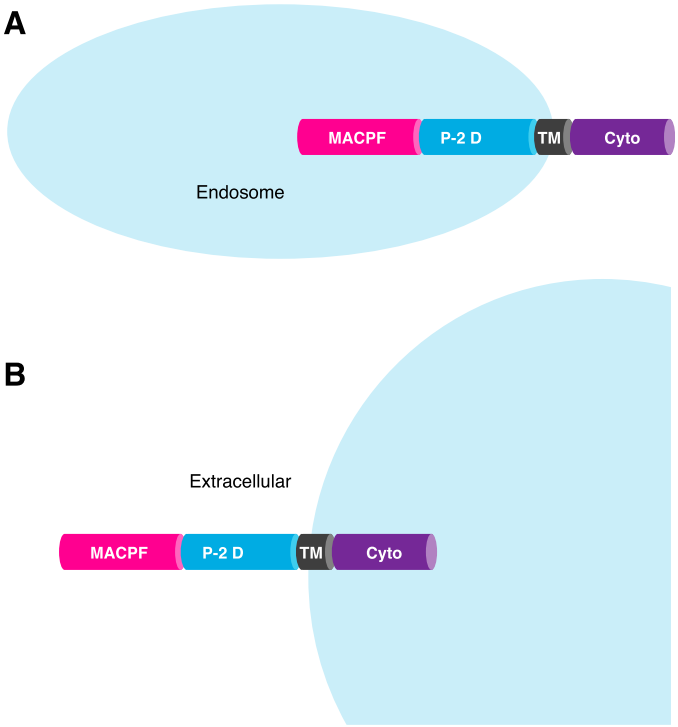

Figure 1. Proposed orientation of Perforin-2 within cells. (A) Orientation of Perforin-2 within an endosome. (B) Orientation of Perforin-2 on the extracellular surface of the cell. P-2 D, Conserved Perforin-2 domain within all organisms that express this protein; TM, transmembrane domain; Cyto, cytoplasmic domain.

This difference in expression kinetics is likely a result of the spread of pathogens from the external tissues to the hemolymph; however, as marine invertebrates are frequently exposed to microbes in their environment, it is also possible that these external organs are primed to respond faster to foreign organisms than the circulating hemocytes.

Unlike sponges, where Perforin-2 was only detectible following bacterial exposure, a low level of Perforin-2 basal expression is observed in abalone epipodia cells and detectible in all organs in oysters [20, 21, 24]. This basal expression suggests that an evolutionary change in Perforin-2 basal expression may have occurred to protect the host better from the environment. Alternatively, this expression could simply result from continuous immune priming with pathogens, resulting in an increased ability to respond to and mount an attack through MACPF polymerization toward minor perturbations in the microbial environment without requiring additional up-regulation of Perforin-2 message.

PERFORIN-2 IN VERTEBRATES

Perforin-2 in vertebrates was first characterized from a differential cDNA screen of human maturing macrophages. As a result of the preferential expression of this gene in terminal stages of macrophage maturation, it was given its initial name of Mpeg1. These initial studies demonstrated expression in primary

macrophages isolated from the peritoneum, spleen, and liver, as well as lower expression levels in well-characterized human macrophage cell lines J774A.1, P388D, M1, and IC-21 [29].

After this initial characterization, Mpeg1 was primarily used in vertebrates as a biomarker for macrophages in human, rodent, and zebrafish models, as a functional role for this protein was not identified [30–35]. Podack and colleagues [36, 37] determined that Mpeg1 is not exclusively localized to macrophages and can be identified in human and murine cell lines and primary cells following stimulation. Given this finding of Mpeg1 in primary cells and nonmacrophage cell lines, Podack and colleagues [36, 37] proposed to rename the Perforin-like protein encoded by Mpeg1 to Perforin-2, given its common MACPF domain. In accordance with previous findings, Perforin-2 is expressed at high basal levels in primary macrophages, as well as macrophage cell lines. In nonmacrophage cells, Perforin-2 protein and message are not present under basal conditions; however, Perforin-2 can be induced to high expression levels following either type I IFN (α, β) or type II IFN (γ) treatment in primary fibroblasts and epithelial cells [36, 37]. Not surprisingly, given previous studies in invertebrates, it was observed that bacterial infection also induced Perforin-2 expression in primary fibroblasts with slightly delayed kinetics when compared with type II IFN treatment alone [36].

The Podack lab [36, 37] determined that Perforin-2 siRNA knockdown abrogated bactericidal activity within cells. We found that Perforin-2 expression correlated directly with bacterial killing against diverse pathogens, including *Salmonella typhimurium, Staphylococcus aureus, Chlamydia trachomatis*, and *Mycobacterium avium*. Likewise, when Perforin-2 was absent from fibroblasts (either from lack of induction or knockdown), bacteria were able to thrive within the intracellular environment [36, 37]. We found that after bacterial infection of fibroblasts expressing Perforin-2, normally resistant bacteria were significantly more susceptible to exogenously added lysozyme. In the absence of Perforin-2 (Perforin-2 knockdown cells or bacteria without Perforin-2-expressing cells), *Mycobacterium smegmatis* and *S. aureus* are resistant to lysozyme's chemical attack. This finding potentially suggests that the MACPF domain of Perforin-2 polymerizes to insert holes within the bacterial surface, allowing lysozyme to gain access to the bacterial peptidoglycan and destabilize the bacteria culminating in lysis. Based on these findings, suggesting that lysozyme has a greater bactericidal effect when Perforin-2 is present, it may be possible that other antimicrobial compounds also use Perforin-2 for effector functions.

Given the ability of Perforin-2 to clear intracellular bacterial pathogens following induction, it was not surprising that *C. trachomatis* is an obligate intracellular pathogen of human epithelia that has evolved mechanisms to be able to suppress Perforin-2 induction actively in epithelial cells [37]. It is likely that this active suppression of Perforin-2 induction is not specific to *Chlamydiae*, and many pathogenic bacteria are also able to suppress Perforin-2 induction through active or passive processes.

Recent manuscripts studying zebrafish have corroborated the importance of Perforin-2 in antibacterial clearance [38, 39]. Interestingly, zebrafish have 2 functional copies of Perforin-2 that are differentially regulated following bacterial infection. Zebrafish also have a 3rd copy of Perforin-2, but it is suggested to be a pseudogene [38]. *Mpeg1* is expressed under basal conditions and is down-regulated following embryo infection with *S. typhimurium* or *Mycobacterium marinum*, whereas *mpeg1.2* is not as readily detectible in basal conditions but is inducible following bacterial infection. Similar to previous invertebrate studies, MyD88 and NF-κB are both implicated as necessary pathways for induction of *mpeg1.2* [20, 38]. Analogous to human and murine in vitro studies, knockdown of gene *mpeg1* or *mpeg1.2* leads to significantly increased bacterial burden in the zebrafish embryo.

The persistent importance of ancient Perforin-2, implicated through evolutionary maintenance, despite the emergence of a multitude of antimicrobial effector molecules, suggests a keystone role for Perforin-2 in antibacterial immune defense. It still remains to be seen if Perforin-2 is critically important for mammalian in vivo defense against pathogens. However, given the strong evolutionary persistence of this protein family, coupled with bacterial infection studies in zebrafish, Perforin-2 could be as important in mammalian in vivo infection models as in vitro models. However, given the layers of redundancy present in the mammalian immune system, it is also possible that the role of Perforin-2 is complemented by redundant mechanisms.

Given that MACPF family members are often equated to being an immunologic "bullet"—triggered to kill—with limited specificity after being released, additional work is necessary to determine what components and signals are involved in the targeting of Perforin-2 and triggering of its polymerization. Given the potentially widespread expression of Perforin-2, improper (excessive) triggering could contribute to major sequelae, ranging from generation of excessive inflammation and autoimmunity, whereas a lack of Perforin-2 triggering could contribute to a host's inability to clear bacterial pathogens, resulting in persistent infection.

PORE-FORMING PROTEINS IN EVOLUTION

Pore-forming proteins are not only present in eukaryotes [40, 41], but also, these proteins exist in bacteria as CDCs, which act primarily as toxins against multicellular hosts. These prokaryotic pore-forming proteins are distantly related to the eukaryotic Perforin-2 protein, thus extending the MACPF superfamily to some of the major Gram-positive toxins. Unlike the bacterial CDCs, the eukaryotic proteins containing a MACPF domain are primarily specific to the immune system. Overall, the MACPF superfamilies' penetrance throughout evolution is fairly complete, except for 3 major phyla in worms, viruses, and archaeal species; however, this lack of identification may, in part, be a result of the in silico algorithms used to detect homology among these dissimilar phyla. Despite this penetrance, not all MACPF domains behave in the same immune-defense role. This is illustrated with *Drosophila's* sole MACPF protein, Torso-like, which has a developmental role in patterning and developmental growth and no recognized immune-related function to date [42, 43].

Amino acid sequences from the MACPF domain of Perforin-2 group into 2 separate clusters in invertebrates and 1 cluster for vertebrates. These clusters are grouped with sponges in 1 cluster and abalone and other invertebrates in the 2nd cluster. These 2 invertebrate clusters share 44% similar and 22% identical amino acids with each other. Interestingly, when the MACPF

domain of sponge Perforin-2 is compared with the human Perforin-2 sequences, there is 46% similarity and 28% identical amino acids. **Figure 2** demonstrates these 3 separate clusters.

Within each cluster, the MACPF domain of Perforin-2 is also highly conserved. For example within the nonsponge invertebrate cluster, there exists a 96% sequence identity between *H. midae* and *H. diversicolor supertexta* and 46% sequence identity between *C. gigas* and *H. corrugata*. Within the vertebrate cluster, there is 65% sequence identity between mouse and human Perforin-2.

Although the MACPF domain is highly conserved, major differences within the Perforin-2 family throughout evolution are apparent in the N-terminal region of the protein. **Figure 3** demonstrates these differences with a phylogenetic tree of the full-length Perforin-2 protein. An example of these differences is observed in the abalone cluster, where a highly conserved furin cleavage site is present at the cytoplasmic domain that is not present in the other clusters [22]. Figure 3 also demonstrates some of the gene duplications that have occurred throughout evolution with the duplication in zebrafish with *Mpeg1*, *Mpeg1.1*, and *Mpeg1.2*, as well as the duplication that occurred in mice with Perforin-2 and PfpI/EPCS50. In zebrafish, both *Mpeg1* and *Mpeg1.1* have been shown to have similar macrophage localizations, whereas *Mpeg1.2* is predicted to be a pseudogene as a result of no observable expression [38]. In mice, Perforin-2 has been demonstrated to be basally expressed in macrophages and has the ability to be induced in other tissues as well; however, the Perforin-2 duplication PfpI/EPCS50 has only been observed in the invading trophoblast [44].

Somewhat surprisingly, given the degree of conservation and otherwise extensive prevalence throughout evolution, Perforin-2

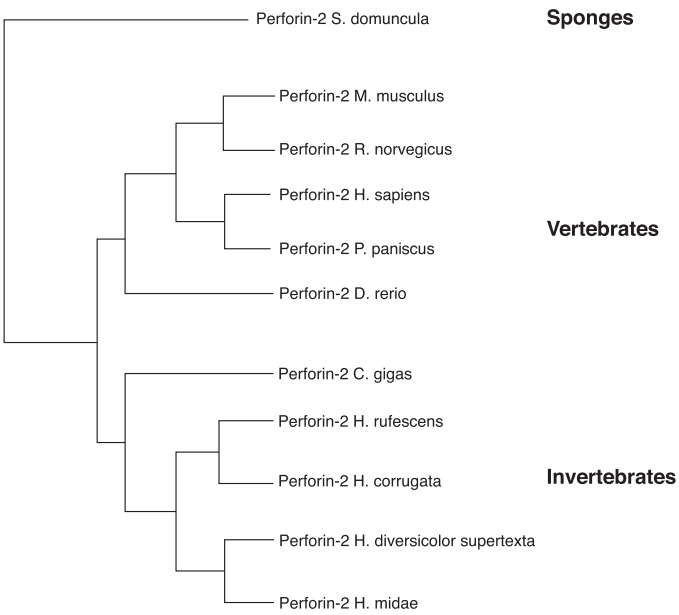

Figure 2. Evolutionary clustering of Perforin-2 MACPF domains. The MACPF domain of Perforin-2 clusters into 3 distinct groups. The 1st is that of sponges, the 2nd is invertebrates, and the 3rd is vertebrates. *S. domuncula*, *Suberites domuncula*; *M. musculus*, *Mus musculus*; *R. norvegicus*, *Rattus norvegicus*; *H. sapiens*, *Homo sapiens*; *P. paniscus*, *Pan paniscus*; *D. rerio*, *Danio rerio*; *C. gigas*, *Crassostrea gigas*; *H. rufescens*, *Haliotis rufescens*; *H. corrugata*, *Haliotis corrugata*; *H. diversicolor supertexta*, *Haliotis diversicolor supertexta*; *H. midae*, *Haliotis midae*.

homologs have not been detected in Drosophila or *Caenorhabditis elegans* genomes. This suggests that Perforin-2 has been lost in protostomes following their divergence [45].

A summary of known Perforin-2 family members is listed in **Table 1**, along with known duplications and pseudogenes. Whereas work is still ongoing to elucidate the mechanism of induction, as well as bactericidal activity, studies in invertebrates, as well as vertebrates, have demonstrated that the Perforin-2 protein is generally induced following bacterial or PAMP exposure and that the protein is bactericidal.

Perforin-2 is exceptionally unique from most other genes in that it exists as an intronless gene in all invertebrates and all vertebrates except for fish. The significance of the lack of an intron is still unknown; however, evolutionary studies by Bird, Trapani, and colleagues [45] postulate that the intron in fish contributed toward a duplication of the fish Perforin-2 gene to produce a common ancestor of Perforin-1 of CTL and NK cells. This work suggests that Perforin-2 may be the modern ancestor Perforin-1 [45].

OTHER MACPF PROTEINS IN VERTEBRATES

Given that the presumed effector domain of Perforin-2 is the MACPF domain, it is necessary to reflect on the mechanism of the MACPF domain from its founding members in the MAC of complement and Perforin-1 (Perforin) of CTL and NK cells [8, 11, 47–51]. Evolutionarily, the MAC of complement evolved with chordates, whereas Perforin-1 evolved with *Gnathostomata*, in particular, with cartilaginous fish [5]. Schematics of Perforin-1 and C9 of the MAC of complement relative to that of Perforin-2 are shown in **Fig. 4**.

The MAC of complement has evolved to attack extracellular bacteria in body fluids. The MAC of complement requires first, initiation through generation of the C5b-C7 initiation complex on the surface of the bacterial pathogen. When this complex is formed, C8 will bind, inducing the attachment and polymerization of 10-16 molecules of C9 to generate the pore-forming structure known as the MAC. Even though C9 leads to the ultimate pore formation, it was the trigger C5b-C7 complex for pore formation in the initiation complex that provided the initial targeting information.

Perforin-1 is expressed by NK and CTL cells and is predominantly used for destruction of virally infected as well as malignant cells. Isolated Perforin-1, containing granules from these cells, demonstrated rapid and nonspecific lysis of cells. In comparison, intact Perforin-1, containing CTL and NK cells, mediates highly specific killing of target cells through precise granule exocytosis through membrane receptors recognizing and binding to the target cell [52]. The granule membrane fuses with the killer cell during exocytosis, thus releasing the contents into the immunologic synapse between the target and killer cells. Unlike complement, the trigger for Perforin-1 polymerization is driven by the release of granule contents (containing Perforin-1) and the presence of extracellular Ca^{2+}.

Given the potentially ubiquitous expression of Perforin-2 in cells, proper targeting is critically important. One major difference between Perforin-2 and the rest of its MACPF family members is that it is the only member that contains

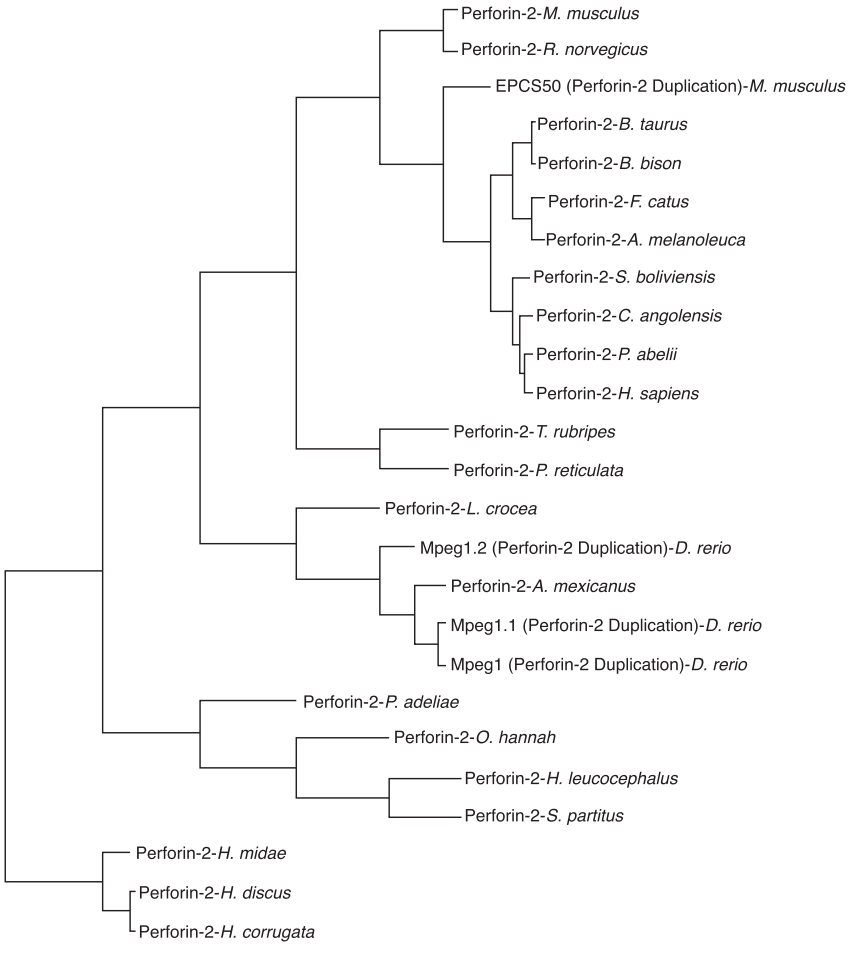

Figure 3. Phylogenetic tree of full-length Perforin-2 protein throughout evolution with known duplications. *B. taurus*, *Bos taurus*; *B. bison*, *Bison bison*; *F. catus*, *Felis catus*; *A. melanoleuca*, *Ailuropoda melanoleuca*; *S. boliviensis*, *Saimiri boliviensis*; *C. angolensis*, *Cordylus angolensis*; *P. abelii*, *Pongo abelii*; *T. rubripes*, *Takifugu rubripes*; *P. reticulata*, *Poecilia reticulata*; *L. crocea*, *Larimichthys crocea*; *A. mexicanus*, *Astyanax mexicanus*; *O. hannah*, *Ophiophagus hannah*; *H. leucocephalus*, *Haliaeetus leucocephalus*; *S. partitus*, *Stegastes partitus*; *H. discus*, *Haliotis discus*.

a transmembrane domain and is not known to be secreted to perform effector functions. This suggests that only the host cell will drive the targeting (as with Perforin-1 in CTL and NK cells) or a combination between host cell and Perforin-2 extracellular targeting molecules could drive polymerization, but this mechanism still needs to be fully elucidated.

CONCLUDING REMARKS

As with other central components of the immune system, pore-forming proteins have maintained a relatively high degree of conservation from one of the earliest multicellular organisms in sponges throughout evolution to modern humans. As with other key components of the immune system, pore-forming proteins classified by a MACPF domain have underwent an expansion throughout evolution. Initially, in early invertebrate animals, such as sponges, only Perforin-2 was present; then, throughout evolution, additional proteins in the MAC of complement and Perforin-1 of CTL and NK cells developed. The high degree of conservation of the initial protein as well as the expansion of this family over time suggest the importance of these MACPF proteins in survival against bacterial and viral pathogens, as well as cancer.

As expected given its conservation, the role of Perforin-2 appears to be largely unchanged between invertebrates and vertebrates. Perforin-2 protein is up-regulated during bacterial infection, and Perforin-2 contributes directly toward the elimination of bacterial pathogens. **Figure 5** is a schematic illustrating the potential trafficking of Perforin-2 to detected foreign organisms. Unfortunately, the subcellular localization of Perforin-2 is currently not well described. As a result of this, the Perforin-2 molecules in the Fig. 5 are present on different vesicles. Yu and colleagues [24] demonstrated in oysters, with the use of an overexpression model, Perforin-2 colocalization with late endosomes; however, additional characterization is still necessary. Figure 5 demonstrates a working model for Perforin-2-dependent killing of intracellular and extracellular pathogens [20, 24, 36–38]. Whereas some invertebrate reports suggest that Perforin-2 may also be involved in antiviral defense, this has not been tested extensively in vertebrates [26]. As a result of the development of the adaptive immune system, Perforin-2's activity against viruses in vertebrates may have become redundant or obsolete.

As the immune system became more complex, a need developed to protect the host against humoral extracellular pathogens. The MAC of complement likely evolved to provide

TABLE 1. Summary of Perforin-2 throughout evolution with expression and functional data

Organism	Expression data	Functional data	Refs.
Sponge: *S. domuncula*	Perforin-2 protein is not basally expressed; protein induction following bacterial PAMP challenge within 24 h	Recombinant protein generated, exhibiting inhibitory activity on bacteria at the nanogram level	[20]
Abalone: *H. rufescens, H. corrugata; H. midae; H. diversicolor supertexta*	Basal expression in gills and epipodia; induction 24–96 h following bacterial infection in epipodia; induction between 48 and 96 h in hemolymph	No clear functional data beyond relative induction following bacterial or PAMP exposure	[21–23, 46]
Oyster: *C. gigas*	Basal expression of Perforin-2 in all tissues; highest basal expression is in digestive glands, gonads, and gills; expression peaks 6 h following bacterial infection in hemocytes and returns to basal levels after 8 h	The MACPF domain of Perforin-2 was purified and incubated with bacteria, exhibiting a dramatic growth induction for gram-positive as well as -negative pathogens	[24]
Zebrafish: *D. rerio*	Three copies of the Perforin-2 gene: *Mpeg1, Mpeg1.1, Mpeg1.2*; *Mpeg1* and *Mpeg1.2* are expressed in macrophages; *Mpeg1.1* is believed to be a pseudogene as a result of no expression; *Mpeg1* is down-regulated during bacterial infection, whereas *Mpeg1.2* is infection inducible	Knockdown of *Mpeg1* results in greater bacterial burden after *M. marinum* infection; knockdown of *Mpeg1* and *Mpeg1.2* also increases bacterial burden following *S. typhimurium* infection	[38, 39]
Mice: *M. musculus*	Two copies of the gene: Perforin-2 and PfpI/EPCS50; Perforin-2 is basally expressed in macrophages and can be induced following IFN treatment in fibroblasts; bacterial infection can induce expression; PfpI/EPCS50 is basally present in the invasive trophoblast cells	Functional data with siRNA knockdown and complementation of Perforin-2 in primary cells as well as cell lines with gram-positive and -negative pathogens; no functional data for PfpI/EPCS50	[36, 37, 44]
Human: *H. sapiens*	Perforin-2 is basally expressed in macrophages and can be induced following IFN treatment in epithelial cells	Functional data with siRNA knockdown and complementation of Perforin-2 in epithelial cells with *C. trachomatis*	[37]

the required humoral antibacterial protection to more complex multicellular hosts. This advancement in humoral immune defense functioned hand in hand with the previously evolved recognition systems, including C3 of complement and other complement activation systems, to provide a cell-free killing mechanism for extracellular bacteria.

Finally, a 3rd MACPF protein in the immune system—Perforin-1—likely evolved from Perforin-2 to eliminate cells that are virally infected or have transformed to cancer [45]. This advancement leads to the ability of the immune system to kill the host's own cells when a perturbation (cancer or viral infection) is detected by CTL or NK cells.

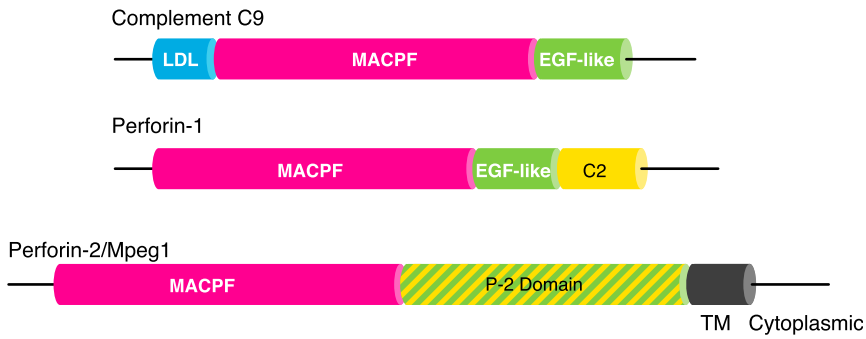

Figure 4. Schematic of C9 of complement and Perforin-1 compared with Perforin-2. Unlike Perforin-2, which is membrane bound, both C9 of complement and Perforin-1 are soluble proteins. EGF, Epidermal growth factor; C2, calcium-dependent plasma membrane-binding domain.

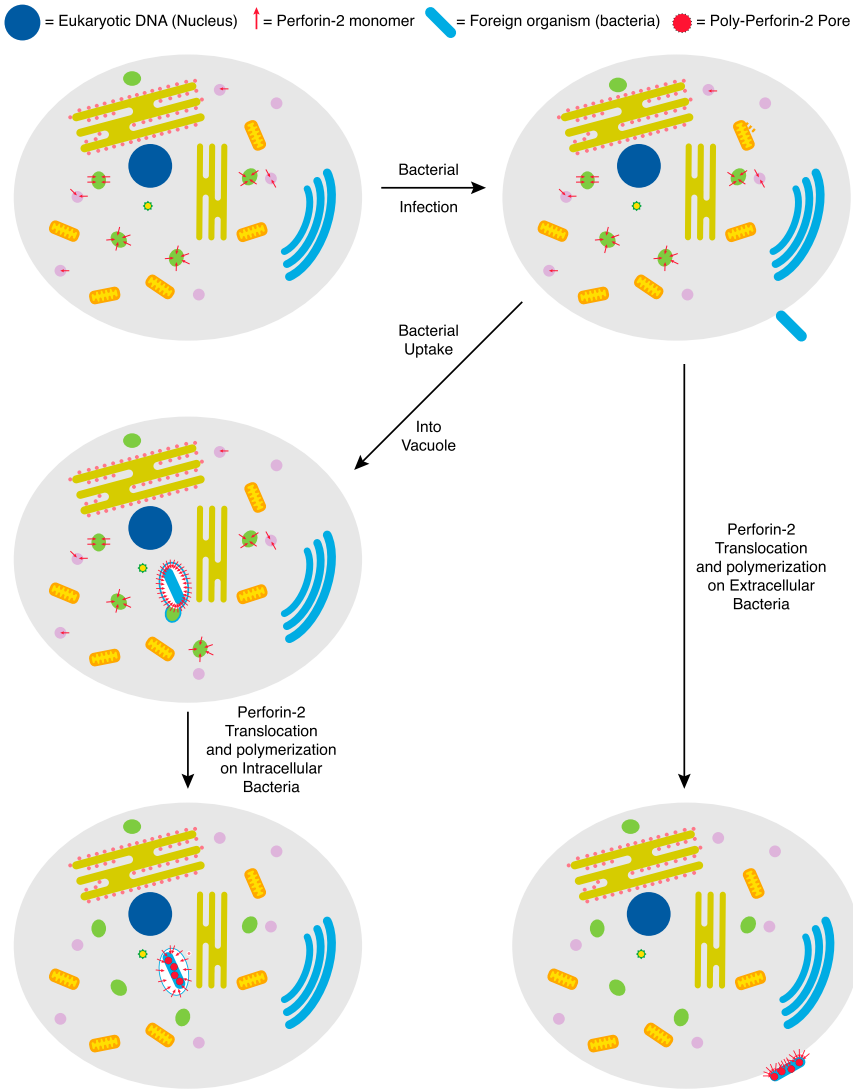

Figure 5. Schematic illustrating the hypothetical model of Perforin-2 trafficking to intra- or attached extracellular foreign organisms and ensuing bactericidal activity.

Each of these MACPF members acts to attack each of the major groups of microbial proteins specifically adapted to its respective target. Likely, the major reason why MACPF proteins are highly selected lies in the use of these proteins in generating access points in the target, enabling other effectors to access key sites in the target and complete the destruction. As shown throughout evolution, this combination of chemical and physical attack via MACPF proteins, along with each protein's respective effectors, is highly efficient.

AUTHORSHIP

R.M. and E.P. designed and wrote this manuscript.

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DISCLOSURES

The authors declare no competing financial interests.

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Perforin-2/Mpeg1 and other pore-forming proteins throughout evolution

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