

HOW PATHOGEN-DERIVED CYSTEINE PROTEASES MODULATE HOST IMMUNE RESPONSES

Sheila Donnelly,^{*,1} John P. Dalton² and Mark W. Robinson¹

¹ *13 Institute (formerly IBID), University of Technology, Sydney, Ultimo, Australia;* ² *Institute of Parasitology, McDonald Campus, McGill University, St. Anne de Bellevue, Quebec, Canada*

**Corresponding Author: Sheila Donnelly—Email: sheila.donnelly@uts.edu.au*

Abstract: In mammals, cysteine proteases are essential for the induction and development of both innate and adaptive immune responses. These proteases play a role in antigen- and pathogen-recognition and elimination, signal processing and cell homeostasis. Many pathogens also secrete cysteine proteases that often act on the same target proteins as the mammalian proteases and thereby can modulate host immunity from initial recognition to effector mechanisms. Pathogen-derived proteases range from nonspecific proteases that degrade multiple proteins involved in the immune response to enzymes that are very specific in their mode of action. Here, we overview current knowledge of pathogen-derived cysteine proteases that modulate immune responses by altering the normal function of key receptors or pathways in the mammalian immune system.

INTRODUCTION

It is the role of the immune system to synthesise and release effector molecules that detect, implement or orchestrate an appropriate response to a potential threat or danger signal. To regulate many aspects of this process, cells of the immune system utilise protease activity. One major group of cysteine proteases, termed cathepsins, are common constituents of the endolysosomal compartments of immune cells where they have critical roles in events such as antigen presentation and zymogen processing. Despite a widely held belief that the cathepsins were enzymes that function only at the acidic pH within the lysosomal compartment, it is now evident that they also operate in the cytosolic

Table 1. Summary of the immuno-modulatory cysteine proteases of pathogenic organisms

Pathogen	Disease	Cysteine Protease	Observed Effect on Immune System
Helminth			
<i>Fasciola hepatica</i>	Fascioliasis	Cathepsin L1	Degrades endosomal TLR3 Cleaves hinge region of IgG
		Cathepsin L2	Cleaves hinge region of IgG
<i>Necator americanus</i>	Helminthiasis	Uncharacterised	IL-4 production by basophils
<i>Paragonimus westermani</i>	Paragonimiasis	Uncharacterised	Cleaves hinge region of IgG
<i>Schistosoma mansoni</i>	Schistosomiasis	Cathepsin B	Inhibits macrophage activation by Th1 stimulants Cleaves hinge region of IgG
<i>Spirometra mansoni</i>	Sparaganosis	Cathepsin S-like protease	Cleaves hinge region of IgG
Protozoan			
<i>Entamoeba histolytica</i>	Amebiasis	EhCp1, EhCp2, EhCp5	Converts Pro-IL-1 β to mature, active IL-1 β Cleaves Pro-IL-18 Degrades C5a and C3a of the complement pathway Cleaves hinge region of IgA1 and IgA2
<i>Leishmania mexicana</i>	Leishmaniasis	Cathepsin B	Induces Th2 cytokine responses Cleaves CD25 from T-cells Inhibition of NF κ B binding to DNA
<i>Plasmodium falciparum</i>	Malaria	PfUCL3	De-ubiquitinase activity—no specific target identified
		PfUCH54	
<i>Toxoplasma gondii</i>	Toxoplasmosis	TgUCL3	De-ubiquitinase activity—no specific target identified
<i>Trypanosoma cruzi</i>	Trypanosomiasis	Cruzipain	Degradation of NF κ B Cleaves hinge region of IgG
Bacteria			
<i>Chlamydia trachomatis</i>	Chlamydia	ChlaDub1	De-ubiquitinase activity—no specific target identified

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Table 1. Continued

Pathogen	Disease	Cysteine Protease	Observed Effect on Immune System
		ChlaDub2	De-ubiquitinase activity— no specific target identified
<i>Escherichia coli</i>	Gastroenteritis	ElaD	De-ubiquitinase activity— no specific target identified
		StcE	Degrades the complement C1-inhibitor serpin
<i>Porphyromonas gingivalis</i>	Periodontal disease	Arg-specific gingipain	Cleaves CD14 from monocytes and fibroblasts
		Lys-specific gingipain	Cleaves IL-6, IL-1Ra, IL-12, TNF α , IFN- γ Cleaves IL-8 Activates complement C3, C4, C5
<i>Prevotella intermedia</i>	Periodontal disease	Interpain A	Activates the C1 comple- ment complex
<i>Streptococcus pyogenes</i>	Pharyngitis	Streptopain (SpeB)	Converts Pro-IL-1 β to mature, active IL-1 β Degrades IgA, IgD, IgG, IgE and IgM Cleaves C3 complement protein
		IdeS	Cleaves hinge region of IgG
<i>Salmonella sp</i>	Salmonellosis	AvrA	De-ubiquitinates I κ B α
<i>Yersinia sp</i>	Yersiniosis	YopJ	De-ubiquitinates TRAF2, TRAF3 and TRAF6 De-ubiquitinates I κ B α
Virus			
<i>Coronaviridae</i>	Gastroenteritis, SARs, common cold	PLP domains	De-ubiquitinates IRF3
Herpes simplex virus 1	Oral and/or genital herpes	UL36	De-ubiquitinase activity— no specific target identified
adenovirus	Respiratory disease, Gastroenteritis	adenain	De-ubiquitinase activity— no specific target identified

and nuclear compartments of cells, as well as the extracellular space.¹ As a result of this widespread localisation, cathepsins can exert diverse effects on the development and regulation of immune responses by playing key roles in cytokine regulation, cell development, induction of apoptosis and influencing Toll-like receptor (TLR) signalling.^{1,2}

Many pathogenic organisms synthesize proteases that resemble host cathepsins (Table 1), both functionally and structurally and have fundamental roles in the life-cycle and survival of the pathogen. For example, parasitic helminths (worms) secrete proteases in large quantities to degrade the extracellular matrix during host invasion,^{3,4} intracellular protozoans require proteases for invasion, nutrition and exit from host cells,⁵ bacterial cathepsins perform housekeeping roles like amino acid uptake and fimbriae maturation^{6,7} and viral proteases are involved in processing of viral gene products.^{8,9} A number of the target amino acid sequences for pathogen cathepsins are also present in key molecules involved in regulating the host immune response including immunoglobulins (Ig), cytokines, TLRs and ubiquitin (Ub). It has been suggested that adaptation of pathogen proteases to target these structures has occurred in an evolutionary process to increase pathogenicity.^{2,10} This has been influenced by the unique biochemical challenges presented to each pathogenic organism and is linked, in part, to their localisation as extracellular or intracellular organisms and therefore their exposure to different cellular structures and macromolecules. Here, we review the functions that have been attributed to pathogen cysteine proteases in the evasion, suppression and modulation of the host immune response.

ADAPTIVE IMMUNE RESPONSES

The immunity and pathology occurring in response to infection with any pathogen is predominantly mediated by T-lymphocytes. In general, control of infection and healing is associated with a polarized Th1 type response whereas the induction of interleukin (IL)-4-dominated Th2 responses are largely suboptimal against a number of pathogens.

Induction of Th2 Immune Responses and/or Suppression of Th1 Responses

In certain protozoan infections, cysteine proteases are critical for the induction of Th2 type immune responses. *Leishmania* are obligate intracellular parasites that live as nonmotile amastigotes within cells of the mononuclear phagocyte lineage of their mammalian hosts. The outcome of infection in leishmaniasis is determined by the Th1 *versus* Th2 nature of the effector response with parasites successfully establishing infection by driving a Th2 immune response.¹¹ Inhibition of *Leishmania mexicana* cathepsin B, with a specific inhibitor, caused a switch in the polarisation of T-cell differentiation from a Th2 to a protective Th1 phenotype in mice.^{12,13} In addition, mutants of *L. mexicana* lacking cysteine protease activity induced less IL-4 and IgE in BALB/c mice compared to wild type parasites.¹⁴ Finally, the delivery of recombinant cysteine proteases derived from either *L. mexicana*, or *Trypanosoma cruzi*, elicited strong Th2-type responses typified by enhanced mRNA expression and production of Th2 cytokines (IL-4, IL-5 and IL-13) from the draining lymph nodes and also a polarised splenocyte response toward a Th2 bias in response to stimulation with anti-CD3.^{15,16}

The ability of cysteine proteases to induce Th2-type immune responses is dependent on their enzymatic activity¹²⁻¹⁷ and also on their substrate specificity as only cysteine proteases belonging to clan CA demonstrate an ability to promote the differentiation of

Th2.¹⁸ Injection of the plant-derived cysteine protease papain to mice was shown to promote the activation of basophils, which then present antigen to CD4⁺ T-cells and induce Th2 cell responses through the secretion of IL-4 and thymic stromal lymphopoietin.¹⁷ Therefore, it was suggested that basophils detect the presence of protease products which in turn activates these cells to induce a Th2 response.¹⁸ This strategy of immune recognition is distinct from typical recognition by cells of the innate system since it is not dependent on the detection of pathogen molecular structures. Instead, it requires the detection of enzymatic activity associated with the presence of a pathogen or antigen. The immune outcome closely resembles the type of reaction that is triggered by helminths and has led to the suggestion that the innate immune system has evolved a detection mechanism based on sensing the abnormal protease activity associated with helminth infection.¹⁸ This idea has support in studies showing that the secreted proteases of the hookworm *Necator americanus* induce Type 2 cytokine production by basophils.¹⁹

Another means by which cysteine proteases could modulate host immune responses towards a Th2 environment may be associated with their ability to inhibit Th1 immune responses. In support of this idea, it was shown that the predominant secreted product of the human and animal helminth pathogen *Fasciola hepatica*, a cathepsin L1 cysteine protease, suppressed the onset of protective Th1 immune responses in mice to infections with the respiratory microbe *Bordetella pertussis*, making them more susceptible to disease. In addition, injection of the cysteine protease immediately prior to immunization of mice with a *B. pertussis* whole-cell pertussis vaccine prevented the development of a Th1 response to the vaccine.²⁰⁻²² Studies with *L. mexicana* cathepsin B suggest that cleavage of CD25 from the surface of T-cells may prevent the development of Th1 responses.¹⁴ Infection with the helminth *Schistosoma mansoni* may provide another example of how cysteine proteases suppress Th1 responses. The acute stages of schistosomiasis leads to the development of a weak Th1 response, but this switches to a potent Th2 response when female worms become fecund and release eggs that get trapped in host liver and intestinal tissues.²³ Secretions from the trapped eggs are responsible for the induction of this Th2 response. The major schistosome proteases, cathepsin B and cathepsin L, are expressed and secreted by the egg stage of the parasite²⁴ and, therefore, may facilitate this immune switching.

INNATE IMMUNE RESPONSES

Substantial evidence supports the view that cysteine proteases secreted by a range of pathogens specifically prevent cells of the innate immune response promoting Th1-adaptive immune responses.^{10,25-27} The mechanisms are varied and depend on the substrate specificity of the protease and of the location of the pathogen within the host.

The innate immune system constitutes the first line of host defence during infection and plays a crucial role in the early recognition of invading pathogens. Unlike the adaptive immune response, the innate immune response is relatively nonspecific, relying on the recognition of evolutionarily conserved structures on pathogens, termed pathogen-associated molecular patterns (PAMPs), through a limited number of pattern recognition receptors (PRRs).²⁸ A number of different PRR families have been described but the best characterised is the family of TLRs. To date, 13 TLRs have been identified in humans and are distinguished by their recognition of distinct PAMPs derived from various pathogens.²⁹

Interaction between a PAMP and its corresponding TLR, present either at the cell surface or intracellularly, leads to the recruitment of an adaptor molecule, followed by the activation of downstream signal transduction pathways.^{28,29} These signaling pathways are classified on the basis of their utilization of different adaptor molecules, i.e., MyD88 or TIR domain-containing adaptor inducing IFN β (TRIF) and, additionally, their respective activation of individual kinases and transcription factors.³⁰⁻³¹ Three major signaling pathways mediating TLR-induced responses have been described (i) NF κ B, (ii) mitogen-activated protein kinases (MAPKs) and (iii) IFN regulatory factors (IRFs). NF κ B and MAPKs are essential in the induction of a proinflammatory response whereas IRFs are required for stimulation of IFN production.³⁰⁻³² Ultimately, TLR-induced signal transduction pathways result in the activation of gene expression and synthesis of a range of molecules such as cytokines, chemokines and immunoreceptors.^{28,30} Together, these coordinate the immediate host response to infection and provide an essential link to the adaptive immune response. Given that the containment and eradication of pathogens is dependent on efficient recognition and signaling through PRRs, it is perhaps not surprising that some pathogens have evolved strategies to interfere with this process.

Degradation of Pattern Recognition Receptors

Among the various bacterial species associated with the development of periodontitis, the Gram negative anaerobic bacterium *Porphyromonas gingivalis* is suspected to be one of the most important causative agents of the chronic form of this disease.³³ Arg- and Lys-gingipain cysteine proteases are the main endopeptidases produced by *P. gingivalis* and are considered to be important virulence factors³⁴ as both proteases inhibit CD14-dependent monocyte activation. CD14 is a 55-kDa glycosylphosphatidylinositol (GPI)-anchored membrane protein, which functions in the detection of many Gram-negative bacteria by cells of the innate immune response. Presentation of CD14-associated bacterial lipopolysaccharide (LPS) to TLR4 and the accessory molecule MD-2, leads to the release of protective inflammatory factors by macrophages and dendritic cells.³⁵ Both Arg- and Lys- specific gingipains from *P. gingivalis* preferentially cleave CD14, but not TLR4, from the surface of macrophages,^{36,37} making the cells hyporesponsive to LPS stimulation. The gingipains display a preference for cleaving CD14 over other polypeptides on human monocytes which can be explained by the high frequency of Arg-X and Lys-X peptide bonds in the amino acid sequence of CD14.³⁸ This preference is further demonstrated by the observation that gingipains also specifically remove CD14 from the surface of gingival fibroblasts.³⁹ As a consequence of this proteolysis, macrophage recognition of the bacterium is attenuated thus neutrophil degranulation and respiratory burst mediated by fibroblast-secreted IL-8 is reduced. Therefore bacterial survival in the periodontal tissues is promoted.⁴⁰

Like the gingipains, the major cysteine protease of *F. hepatica*, cathepsin L1, is secreted into the extracellular environment during infection and also inhibits macrophage recognition of bacterial products. However, this inactivation is not mediated by cleavage of cell surface CD14. Instead, cathepsin L1 is internalised by the host's phagocytic macrophages and trafficked to the endolysosomal compartments where it specifically degrades TLR3.²⁵ This variation in proteolytic activity reflects clear differences in substrate specificity between the Arg-/Lys-specific gingipains and cathepsin L1 which prefers hydrophobic residues such as Leu, Phe and Ala.^{41,42} Considering both CD14 and

members of the Toll receptor family contain multiple leucine-rich repeat motifs⁴³ it is perhaps surprising that cathepsin L does not cleave either CD14 or TLR4 from the surface of macrophages yet specifically degrades intracellular TLR3. Unlike the surface Toll receptors, TLR3 undergoes conformational changes in response to lysosomal acidification⁴⁴ which may make it susceptible to cleavage by cathepsin L, a protease that is stable and functional over a broad pH range.⁴¹

In general, macromolecules internalized by macrophages are degraded into antigenic peptides by the range of endogenous cathepsins resident in the endolysosomal compartments. However, *F. hepatica* cathepsin L1 is resistant to this endosomal degradation and data shows that the mature enzyme is highly resistant to proteolytic degradation by various endopeptidases.⁴² It has also been reported that *F. hepatica* cathepsin L1 can degrade cystatins/serpins such as SCCA1 and SCCA2⁴⁵ which may protect the enzyme from inhibition by cystatins within the lysosome that are known to regulate the activity of resident endolysosomal cathepsins.⁴⁶

During schistosomiasis, the eggs released by *S. mansoni* suppress the maturation of dendritic cells in response to the bacterial products poly-I:C and LPS.⁴⁷ Similar to *F. hepatica* cathepsin L1, immature dendritic cells rapidly internalized egg antigens and targeted them to endolysosomal compartments.⁴⁸ The egg antigens contain cysteine proteases cathepsin B and cathepsin L which, like *F. hepatica* cathepsin L1, may cleave TLRs within the endosome thus preventing the dendritic cells from maturing in response to activation signals.²⁵ This in turn would inhibit Th1 responses and allow the promotion of Th2 responses which correlates with the immune switching during egg deposition.

Inhibition of NF κ B Signalling

Several studies have demonstrated that eradication of the intramacrophage-dwelling *Leishmania sp.* requires the induction of Th1 cells. Prolonged survival of these pathogens is associated with the parasites ability to regulate IL-12 production by macrophages and therefore control the production of protective IFN- γ .⁵⁰⁻⁵⁴ Most mouse strains are resistant to infection with *L. major* but develop nonhealing lesions following infection with *L. mexicana*.²⁰ The inability of these mouse strains to heal following infection with *L. mexicana* is associated with a higher level of parasite cysteine protease B activity and subsequently lower induction of IL-12.⁵⁵ This role for the parasite protease was confirmed by the observation that amastigotes of protease deletion mutants of *L. mexicana* had limited ability to inhibit IL-12 production and unlike the wild type parasites, were unable to suppress a Type 1 adaptive immune response.⁵⁵ The mechanism of action appears to be the specific proteolytic degradation of NF κ B, which did not affect the nuclear translocation of this transcription factor, but did prevent it binding to DNA and therefore inhibited its ability to induce IL-12 gene expression.⁵⁶

The infection of cells with the obligate intracellular protozoan parasite, *Toxoplasma gondii* also results in the inhibition of IL-12 expression via interference with NF κ B activation. In this case the termination of NF- κ B activity was associated with a reduction of the phosphorylation of p65/RelA, an event required for the translocation of NF- κ B to the nucleus.⁵⁷ While a number of cysteine proteases have been identified from *T. gondii*,⁵⁸ the possibility of their involvement in this immune-modulatory effect has not been investigated.

Prevention of Ubiquitination

Following interaction between a PAMP and its corresponding PRR, the activation of downstream signal transduction pathways is dependent on the ubiquitination of protein components of the signalling cascade.^{30,31} Ubiquitination is an enzyme-mediated process by which ubiquitin (an 8 kDa protein) is covalently attached to lysine residues of target proteins. While this alteration to proteins does not mediate degradation it has functional consequences for the modified protein such as changes in their conformation, subcellular localization or catalytic activity. However, ubiquitination is a reversible modification and the rapid removal of ubiquitin from substrates is catalysed by de-ubiquitinating enzymes (DUBs) which are predominantly classed as cysteine proteases. The human genome is predicted to encode almost 500 proteins that are known to recognize ubiquitin and attach it to specific substrates and over 90 DUBs that reverse that reaction.⁶⁰

During virus replication, the innate immune response is activated, resulting in the production of several hundred antiviral proteins converting the intracellular environment into a suboptimal context for replication.⁶⁰ In response to this selective environment, evidence suggests that a number of viral proteases have adapted a ubiquitin-removal specificity as a mechanism to disable the host immune system and optimize the intracellular environment for efficient virus replication and release.¹⁰ For example, the nonstructural protein of severe acute respiratory syndrome (SARS) corona virus, nsp3, known to be involved in the processing of replicase polyproteins, has recently been shown to carry a conserved deubiquitinase (DUB) motif within its papain-like protease (PLpro) domain.^{61,62} This protein efficiently inhibits IRF3 ubiquitination in a protease dependent mechanism.⁶³ IRF3 is a critical transcription factor for the activation of antiviral IFN and requires ubiquitination to translocate to the nucleus. Its inhibition by a viral DUB could explain why cultured cells infected with *Coronaviridae* characteristically produce very low levels of IFNs.⁶⁴⁻⁶⁶ Indeed, most viruses, including all highly pathogenic human viruses, attempt to modulate this aspect of the innate immune response early in infection.^{66,67}

Virus-encoded DUBs from a variety of virus families have been described,¹⁰ including UL36 of herpesviruses such as herpes simplex virus Type 1, Epstein-Barr virus and mouse and human cytomegalovirus,⁶⁸⁻⁷⁰ the adenain protease of adenovirus,⁷¹ and the PLP domains from *Coronaviridae*.⁷² These proteases are unique to the viral pathogens and quite distinct from host-encoded DUBs. While a pathogenic role has not been assigned to many of these viral DUBs, it is tempting to speculate that the prevention of ubiquitination of essential transcription factors is a common mechanism to suppress antiviral IFN production.

Despite having no intrinsic ubiquitin system, several bacterial strains have been found to contain ubiquitin-specific cysteine proteases. Like the viral DUBs, these proteases interfere with innate cell signalling, inhibiting activation of antibacterial responses by specifically targeting elements of the NF κ B pathway. For example, YopJ secreted by *Yersinia sp.* was first described as a DUB, preventing ubiquitination of the TLR-adaptor proteins TRAF2, TRAF3 and TRAF6.^{73,74} It has also been reported that YopJ can de-ubiquitinate the transcription factor I κ B α , thereby preventing its degradation and the subsequent translocation of NF- κ B to the nucleus.⁷⁵ Similarly, AvrA, a protease secreted by *Salmonella sp.* stabilises I κ B α by preventing its ubiquitination and thereby inhibits NF- κ B activated inflammatory responses of the host.⁷⁵ De-ubiquitinase activity has also

been attributed to cysteine proteases secreted by *Chlamydia trachomatis* (ChlaDub1 and ChlaDub2)⁷⁷ and *Escherichia coli* (ElaD)⁷⁸ although a precise role has not yet been defined.

Bioinformatic analyses have predicted that a number of medically-relevant parasitic protozoa encode putative DUBs.⁷⁹ However, only three have been functionally assessed for their ability to bind to ubiquitin. *Plasmodium falciparum* (the causative agent of malaria) expresses two DUBs, namely PfUCHL3 and PfUCH54⁸⁰ and a third, TgUCHL3, a homologue of PfUCHL3, is expressed by *T. gondii*.⁸¹ It has been hypothesized that these proteases may only function within the parasite itself and not target host proteins. There is currently no information on their physiological effects.

DEGRADATION OF SOLUBLE MEDIATORS OF IMMUNE RESPONSE

Degradation of Cytokines and Their Receptors

Cells of the innate and acquired immune systems communicate via the release of cytokines. These proteins are released in response to the presence of pathogens or to components of damaged tissue and are essential for the induction of an inflammatory response as well as its regulation and resolution. Interruption of this communication network profoundly impacts on the outcome of infectious disease. One method of disruption of signalling, performed promiscuously by cysteine proteases derived from *P. gingivalis*, occurs through the proteolytic modification of cytokines (IL-6, IL-1Ra, IL-12, TNF- α , IFN- γ) and their receptors (IL-6R).²⁷

A second method of interference by pathogen proteases is mimicking the activity of host proteases used in the regulation of cytokine activity. Unlike most other cytokines, IL-18 and IL-1 β lack a signal peptide and are first synthesized as biologically inactive precursors (proIL-18 and proIL-1 β). These precursors are cleaved by caspase-1 (IL-1 β -converting enzyme [ICE]), between Asp-116 and Ala-117^{82,83} to produce the mature active cytokines. Cysteine proteases isolated from *Streptococcus pyogenes* (SpeB) and *Entamoeba histolytica* both convert Pro-IL-1 β to a mature cytokine, targeting cleavage sites 1 and 5 amino acids from the caspase-1 site of action, respectively.^{84,85} In both cases the resultant cytokine retains biological activity which leads to the augmentation of an inflammatory response. For *E. histolytica* this action may facilitate its spread beyond cells in direct contact with amoebic trophozoites.⁸⁴ In addition, the ICE-like activity of pathogen cysteine proteases suggests a mechanism which could activate caspases within infected cells and thus induce cell death by apoptosis.

Another cysteine protease from *E. histolytica* cleaves pro-IL-18.⁸⁶ However, the proteolytic action removes Glu-42, a key residue for biological activity of IL-18,⁸⁷ and therefore, the cleavage product is an inactive protein. It was suggested that the secondary structure of IL-18 may contribute to the choice of cleavage site, as the amino acid sequence does not correlate with known peptide substrate specificities.⁸⁷

Effect on Chemokines

Chemokines are a distinct, large superfamily of cytokines encompassing small structurally-related proteins. Physiologically, chemokines have an important role in the recruitment of leukocytes during an acute inflammatory response. Host proteases tightly regulate this cellular recruitment by modulating the activity of chemokines via specific

cleavage of their N- and C-termini.^{88,89} The proteolytic cleavage of the N-terminus enhances activity for several CXC inflammatory chemokines, including the main neutrophil-attracting chemokine CXCL8 (IL-8). This function has been appropriated by the gingipains of *P. gingivalis* which efficiently cleave IL-8 at the N-terminus, enhancing its activity,⁹⁰ an action which correlates with the massive infiltration of neutrophils observed at sites of periodontitis. However, when associated with bacterial outer membrane vesicles the same proteases can also degrade IL-8. It has been suggested that this dual role of enhancing and inhibitory activity results in the association of pro- and anti-inflammatory reactions to distal and proximal positions of the bacterial plaque, respectively, and explains why there is no elimination of infection despite the accumulation of neutrophils.⁹⁰

Degradation of Immunoglobulins

Immunoglobulins (Igs) play a key role in host immune defence mechanisms by specifically recognising invading organisms and mediating their killing by professional phagocytes or the complement system, or both. Igs are composed of antigen-recognising Fab regions, linked through a flexible hinge region to a constant Fc effector region. The Fc section interacts with Fc receptors on phagocytic cells and triggers the activation of the classical pathway of complement. The classical pathway is initiated by the binding of complement factor C1q to specific IgG or IgM. This interaction triggers a cascade of events resulting in the opsonisation of antigen with C3b forming an immune complex which is efficiently recognized by phagocytes.

Despite the fact that mammalian hosts infected with the helminth *F. hepatica* develop specific antibodies⁹¹ and that the major protein isolated from eosinophils is highly toxic to newly excysted juvenile (NEJ) worms,⁹² no evidence exists of antibody-mediated eosinophil damage to NEJs in nonpermissive bovine hosts.⁹³ While effector cells readily adhered to NEJs in the presence of immune sera, they failed to adhere if the parasite's excretory/secretory (ES) products were added which indicated that the contents of ES were preventing interaction between immune sera and eosinophils. In the presence of leupeptin, the effector cells remained attached to the NEJs, suggesting a role for cysteine proteases.⁹⁴ Subsequently, *in vitro* studies have demonstrated that the papain-like cathepsin L1 and L2 proteases secreted by *F. hepatica* cleaved all human IgG subclasses in the hinge region. Despite clear evidence that these proteases have distinct peptide bond preferences, both cathepsin L1 and L2 cleaved each of the IgG molecules at the same peptide bond.⁹⁵

The ability to degrade human IgG subclasses is not exclusive to the cathepsin Ls of *Fasciola*. Similar proteolytic cleavage sites and specificity for human IgG subclasses have been shown for the major secreted cysteine proteases of other helminths, such as *Paragonimus westermani*,⁹⁶ *Spirometra mansoni*⁹⁷ and *Schistosoma mansoni*⁹⁸ which all degrade host IgG *in vitro* and prevent parasite specific antibody-mediated eosinophil activation.⁹⁹ Cruzipain, the cysteine protease secreted by the protozoan parasite *T. cruzi* also exhibits cathepsin-like activity, cleaving all human IgG subclasses at the hinge region in a similar but not identical region to cathepsins L1 and L2 of *F. hepatica*.¹⁰⁰ The fact that the main cleavage sites for this range of proteases all exist within the hinge regions of IgG, suggests that the conformation of the antibody molecules influences the accessibility of enzymes and therefore determines the specificity of cleavage, irrespective of the amino acid preference of each protease active site.

In all cases, the end result of proteolytic cleavage by these parasite proteases is the release of intact monomeric fragments of Fab.^{95,98,100} The Fab fragments retain their capacity

to bind to surface antigens, a term called 'fabulation'. Surface epitopes are therefore masked from intact and functional antibodies, while the loss of Fc fragments eliminates the interaction of IgG with effector cells and prevents resultant antibody-dependent cytotoxicity. In addition, the Fc fragments produced by initial proteolytic cleavage at the hinge region are further degraded by proteases from *F. hepatica* and *T. gondii* cruzipain in the CH2 region producing Fc-like-fragments of 14 kDa composed of the CH3 domain.^{95,100} As the complement factor C1q specifically binds to the CH2 region of IgG, removal of this would prevent the initiation of the classical pathway.

Despite lacking sequence identity to the helminth papain-like proteases, the cysteine protease (IdeS) secreted by the pathogenic bacterium *S. pyogenes* adopts a canonical papain fold¹⁰¹ and like papain, cleaves IgG in the flexible hinge region of the IgG heavy chain generating intact Fab and Fc fragments.¹⁰² Similar to the outcome with helminth-specific immune responses, survival of opsonised bacteria against the phagocytic actions of macrophages or polymorphonuclear leukocytes is significantly enhanced in the presence of IdeS, due to the proteolytic interference of Fc-mediated killing.¹⁰³ Amongst the microbial secreted cathepsins, IdeS is unique in its specificity for IgG, with no additional substrates identified to date. The other major cysteine protease secreted by *S. pyogenes*, SpeB, is structurally homologous to papain and cleaves IgG in the flexible hinge region, although at a different cleavage site.¹⁰⁴ However, the proteolytic activity of SpeB towards immunoglobulins is not restricted to IgG. This protease also degrades the carboxy-terminal of the heavy chains of IgA, IgD, IgE and IgM.¹⁰³

Specific-IgA protease activity is a well-established feature of many human infectious diseases that take place at, or originate from, mucosal surfaces.^{105,106} For bacterial pathogens the IgA proteases have been primarily classified as serine or metallo-type proteases cleaving only the IgA1 subclass of antibody, because the susceptible site is one of the Pro-Ser or Pro-Thr peptide bonds located within a 12-amino acid proline rich sequence in the hinge region of IgA1 but absent from IgA2.^{106,107} However, the cathepsin B-like cysteine protease (EhCp5) secreted by the protozoan *E. histolytica* with a preference for Arg-Arg residues displays an ability to cleave both IgA1 and IgA2 degrading the antibody structures at positions 245 and 250 of the hinge region.^{108,109} Similar to the proteolytic cleavage of IgG described above, release of Fab fragments inhibits antigen disposal as immunogenic determinants are masked by fabulation. Moreover, removal of the Fc region eliminates the ability of IgA to agglutinate, preventing opsonophagocytosis thus facilitating survival of pathogenic organisms at the mucosal surface.

Inactivation of Complement Pathways

The complement system is composed of three distinct pathways: (1) classical pathway, activated by antigen-antibody complexes; (2) lectin pathway, activated by carbohydrate arrays found on microbial surfaces; and (3) alternative, activated by C3 binding to the surface of micro-organisms. Central to all pathways of activation is the formation of C3 and C5 convertase complexes. In particular, the C3 protein and its activated form, C3b, is an integral component of the C5 convertase complex whichever pathway of activation has been engaged. Cleavage of these molecules produces the C3a and C5a fragments which are responsible for attracting white blood cells to the site of infection and therefore form a link between the innate and adaptive immune systems.

Of the members of the complement cascade, cysteine proteases secreted by pathogens primarily target components of the C3 and C5 convertase complex. A number of bacterial proteases, including the SpeB cysteine protease of *S. pyogenes* cleave C3 and thus inactivate or prevent the formation of the C5 convertase complex.^{110,111} Similarly, the cysteine protease from *E. histolytica* prevents the formation of the membrane attack complex (MAC) and the release of the pro-inflammatory mediator, C5a by degrading both C5a and C3a directly.^{109,112}

The refractoriness of *P. gingivalis* to the immune response has been attributed to the biphasic effects of gingipains on the complement system. The lysine-specific gingipain is capable of degrading the C5a receptor, but more importantly the arginine-specific enzymes cleave C3, C4 and C5.¹¹³ Sharing substrate specificity with the proteases required for complement activation, the gingipains activate C3, C4 and C5 by cleaving them at their activation sites. However, at high concentrations the Arg-gingipains completely degrade and thus inactivate the complement proteins.¹¹³ This has led to the suggestion that early infections by *P. gingivalis* may actually stimulate complement activation to cause an inflammatory state that is advantageous to the bacterium. However, as infections become chronic the higher number of bacteria lead to the degradation of complement. This idea is supported by data showing that the cysteine protease, Interpain A, secreted by the periodontal bacterium, *Prevotella intermedia*, which co-aggregates with *P. gingivalis*, activates the C1 complex in serum, causing deposition of C1q on bacterial surfaces and resulting in a local inflammatory reaction during the initial stages of infection.¹¹⁴

Activation of the complement cascade involves a number of proteases which are tightly regulated by cystatins and serpins. The StcE cysteine protease of *E. coli* uniquely prevents complement activation via the classical pathway by specifically cleaving the serpin, C1-inhibitor, which regulates the proteases of the initiating C1 complex of the classical pathway.¹¹⁵

CONCLUSION

The immune system has evolved many different strategies to control many types of infections and thus prevent associated disease. Equally, pathogenic organisms appear to have developed processes to evade, suppress or subvert the immune response with pathogen-derived cysteine proteases emerging as key molecules. Whilst some of these proteases share a common origin with mammalian-encoded proteases, most of them have ancient intrinsic functions, such as processing pathogen protein components and may have acquired the specificity for host protein targets by interaction with their host's immune system over time. As many of these proteases have evolved distinct biochemical features from their mammalian counterparts they remain attractive as targets for new antimicrobial drugs. In addition, some of these proteases may be useful as novel therapeutics for the treatment of Th1 inflammatory disorders particularly in light of the current strategy of developing antagonists of innate immune responses as immunotherapeutics for sepsis and autoimmune disease.

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