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REVIEW

Intestinal proteases of free-living and parasitic astigmatid mites

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Abstract Among arthropod pests, mites are responsible for considerable damage to crops, humans and other animals. However, detailed physiological data on these organisms remain sparse, mainly because of their small size but possibly also because of their extreme diversity. Focusing on intestinal proteases, we draw together information from three distinct mite species that all feed on skin but have separately adapted to a free-living, a strictly ecto-parasitic and a parasitic lifestyle. A wide range of studies involving immunohistology, molecular biology, X-ray crystallography and enzyme biochemistry of mite gut proteases suggests that these creatures have diverged considerably as house dust mites, sheep scab mites and scabies mites. Each species has evolved a particular variation of a presumably ancestral repertoire of digestive enzymes that have become specifically adapted to their individual environmental requirements.

Keywords Protease · *Sarcoptes scabiei* · *Psoroptes ovis* · *Dermatophagoides pteronyssinus*

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Introduction

With an estimated 48,000 species described by the turn of the century (Halliday et al. 2000) and a total estimate of between 0.5 and 1 million species, the mites are one of the most diverse groups of living organisms. Their diversity is paralleled by the wide array of habitats that they exploit and by the diversity of recent research undertaken to investigate them. Areas that are covered in greatest depth are acarid crop pests and medically or veterinary important mites causing human and livestock diseases. Among the latter are three important groups of astigmatid mites: house dust mites, which are free-living but feed on shed human skin scales; sheep scab mites (*Psoroptes ovis*, family Psoroptidae), which are adapted to feeding on the skin surface merely abrading its outer layer; and scabies mites (*Sarcoptes scabiei*, family Sarcoptidae), which burrow into the skin. Although *S. scabiei* has been known as a parasitic burden to humans and livestock since the earliest scientific records (for a review, see Burgess 1994) and *P. ovis* results in considerable economic losses among farmers of cattle, sheep and goats, probably the best known acarid pathogens today are the house dust mites of the family Pyroglyphidae (particularly *Dermatophagoides pteronyssinus*, *D. farinae* and *Euroglyphus maynei*) and the super family Glycyphagoidea (particularly *Blomia tropicalis*). About 10 %–15 % of the human population are estimated to suffer from allergies caused by the faecal pellets of these ubiquitous creatures (Basagana et al. 2004). More than 95 % of mite allergens are associated with the faecal pellets and are not direct components of the mite body (Tovey et al. 1981), indicating that the allergens of importance are in fact enzymes that are presumably associated with digestive processes (Stewart et al. 1989). Coincidently, the homologous parasitic mite proteins of the gut proteases of house dust mites also seem to be important in pathogenesis. Most importantly, mite

gut proteases are probably essential to mite survival, as they allow the acquisition of nutrients and facilitate the host tissue invasion and the evasion of host immune defence mechanisms. Thus, mite gut proteases are promising targets for the development of novel chemotherapeutic, immunotherapeutic and serodiagnostic agents. The focus of this review is to compare the significant diseases caused by these three mite groups, highlighting some of the currently known repertoire and functional characteristics of their gut proteases.

Mite biology and significance in disease

House dust mites

House dust mites are free-living in the environment, feeding on shed skin scales and other human detritus. Adult mites are approximately 0.5 mm in length. Mature adult females lay 1–2 eggs per day that undergo incomplete metamorphosis through larvae, protonymph, tritonymph and adults. Adult female mites live for around 2 months depending on environmental conditions with the highest mite survival being seen under conditions of high humidity and a warm temperature. In many regions of the world, house dust mite species of the Pyroglyphidae family and Glycyphagoidea super-family are the most common source of indoor allergens (for a review, see Thomas et al. 2010). The most important species are the pyroglyphid mites *D. pteronyssinus* and *D. farinae* and to a lesser extent *Euroglyphus maynei* and the glycyphagid mite *Blomia tropicalis*. *D. pteronyssinus* and *D. farinae* are both abundant in many regions; however, in Australia and UK, *D. pteronyssinus* predominates. *Blomia tropicalis* is the dominant species found in many parts of Asia including Malaysia (Mariana et al. 2000), Sinagpore (Chew et al. 1999b) and Hong-Kong (Sun et al. 2004).

Significance as a cause of allergy and interaction with the epithelium

An estimated 10 %–15 % of individuals in the western world suffer from asthma (Basagana et al. 2004). Of these, approximately 85 % are thought to have an allergy to house dust mites (Thomas et al. 2010). Asthma is the most common chronic inflammatory disease in children. It has been extensively studied and shown to result in reversible obstruction of the airways, airway hyperresponsiveness and remodelling and mucus hypersecretion. Infiltration of the airway submucosa with either neutrophils or eosinophils also often occurs (Fajt and Wenzel 2009).

Many of the reported house dust mite allergens are enzymes involved in mite digestive processes (Stewart et al. 1989; Arlian and Morgan 2000; Arlian 2002). The major source of house dust mite allergens are mite faecal pellets (Tovey et al. 1981), which can become aerosolised and are inhaled. The faecal pellets are rich in protein and consist in food, debris and digestive enzymes covered in a chitinous peritrophic membrane (Gregory and Lloyd 2011). House dust mites can re-ingest their own faecal pellets several times to achieve further digestion of the nutritional content. A single mite can produce over 2000 faecal pellets and even more partially digested pellets containing digestive enzymes (Gregory and Lloyd 2011).

Exposure to house dust mite allergens induces a complex innate and adaptive immune response in the airway epithelium via mechanisms similar to those observed in allergic rhinitis and dermatitis (for a review, see Jacquet 2011). Bronchial epithelial cells play a central role in the response encompassing the instruction of the pulmonary dendritic cells to induce a Th2 response via release of pro-Th2 cytokines including granulocyte-macrophage colony-stimulating factor, interleukin-25 (IL-25) and IL-33 (for a review, see Gregory and Lloyd 2011). A characteristic infiltration also occurs by eosinophils, degranulated mast cells and Th2 lymphocytes producing cytokines such as IL-4, 5, 9 and 13. Studies have demonstrated that the biological functions of many house dust mite allergens can amplify their allergenic nature (for a review, see Jacquet 2011).

Sheep scab mites

Sheep scab, caused by infestation with *P. ovis* is, arguably, the most important ectoparasitic disease of sheep in the UK in terms of both animal welfare and financial impact. The disease is endemic in both hill and lowland sheep in all areas of the British Isles, with an estimated 7000 outbreaks in 2004 (Bisdorff et al. 2006). In continental Europe and in the USA, psoroptic mange is also a welfare problem in cattle and is becoming increasingly common in the UK (Jones et al. 2008). Sheep scab is highly contagious, causing considerable pruritis and irritation and represents a major welfare concern (Kirkwood 1986; van den Broek and Huntley 2003). Current disease control strategies are reliant upon chemotherapy; however, the emerging issues of residues, eco-toxicity and parasite resistance have raised concerns regarding the sustainability of current control strategies (van den Broek and Huntley 2003; Nisbet and Huntley 2006).

Clinical features and transmission

The life cycle of *P. ovis* is carried out exclusively on the ovine host, taking 11–19 days from egg hatch to egg production by the adult (Sweatman 1958; van den Broek and Huntley 2003). Mites can survive off-host but only remain infective for 15–16 days once removed (O'Brien et al. 1994;

van den Broek and Huntley 2003). The mites themselves are visible to the naked eye, with adult females being approximately 1 mm in length and 0.5 mm across. Sheep scab is characterised by exuberant yellowish scabs, with additional signs including restlessness, scratching, yellow-stained fleece, wool-loss, head tossing, bleeding wounds and loss of condition (van den Broek and Huntley 2003). In extreme cases, the disease can be highly debilitating causing a high degree of morbidity and, although rare, some animals can suffer from epileptiform-like seizures that can be fatal (van den Broek and Huntley 2003). Sheep scab is most commonly spread by direct contact with an infested animal or by contact with live mites in wool tags or on fences or farm machinery. The skin lesions associated with the disease are induced by mite-derived pro-inflammatory factors, a likely source of which are mite excretory/secretory products, including potent enzymes and allergens.

Disease progression is characterised by three distinct phases: early and late phases and a later decline phase. The early phase is associated with low mite numbers and small lesions of no greater than 2 cm across. These small lesions are extremely difficult to locate and make early disease diagnosis a challenge. During this time, mites adapt to the new environment and the host becomes sensitised to mite allergens deposited on the skin. This early stage can last from 2–3 weeks and few clinical symptoms are observed during this time (van den Broek and Huntley 2003). During the late phase, a rapid increase in mite numbers is observed as the lesion spreads across the body of the sheep; in extreme cases, lesions can cover most of the skin. The heat and humidity produced by the inflammatory response within the fleece provides an ideal microclimate for mite survival. A phenomenon known as "flaker" sheep might occur at this time with extensive wool loss with denuded areas of skin being covered in flaky scabs, overlying thousands of active mites (van den Broek and Huntley 2003). These cases are more reminiscent of crusted scabies in humans but the underlying mechanisms or genetic predispositions involved are poorly understood. Because of the intense itching and high numbers of mites present, mites are most likely to be transmitted to other sheep during the late phase. Finally, the disease enters a decline (or recovery) phase with the active leading edge of the expanding lesion becoming less distinct. At this time, the mite population starts to decline because of a lack of available feeding sites (naive skin) and the increasing role of the host protective immunity. Following the decline phase, many animals make a full recovery, with new wool growing on the previously denuded areas as the scab lifts away from the skin. Some sheep appear to recover completely but can still harbour populations of mites either under dried scabs or in the so-called cryptic sites such as the ear (van den Broek and Huntley 2003).

Interaction with host epidermis

P. ovis is a non-burrowing surface-exudate feeder capable of consuming serous fluids, lymph and erythorocytes (DeLoach and Wright 1981). Mites survive on the surface of the skin and their mouthparts (pseudorutella) do not appear to penetrate beyond the outermost layer of the skin, namely the stratum corneum (Mathieson and Lehane 2002). The mites abrade the stratum corneum and deposit allergens as they progress. The combination of mechanical skin abrasion, mite allergen deposition and increased self-grooming behaviour by the host in response to the pruritis caused by the mites triggers the subsequent activation of a cutaneous inflammatory reaction (van den Broek and Huntley 2003; Burgess et al. 2010) providing an exudate that supplies the mite with a food source (Hamilton et al. 2003). Terminally differentiated keratinocyte cells, termed corneocytes, within the stratum corneum are the first point of contact between the parasite and the host immune response. Therefore, the establishment of a *P. ovis* infestation is the result of a complex interaction between the host and the mite, during which the mite appears to initiate reactions conducive to its own establishment and maintenance (Sinclair and Filan 1991). Previous transcriptomic analysis of the host skin response to infestation with *P. ovis* has demonstrated the differential expression of over 1500 genes over a 24-h time course. The analysis has implicated a number of genes with roles in allergy and inflammation, including pro-inflammatory cytokines, i.e. *IL-1A*, *IL-1B*, *IL-6*, *IL-8* and *tumour necrosis factor* and factors involved in immune cell activation and recruitment, i.e. the selectin genes *SELE*, *SELL* and *SELP* and *intercellular adhesion molecule 1*, *colony stimulating factor 2* (*CSF2*), *CSF3*, *chemokine (C-C motif) ligand 2* and *chemokine (C-X-C motif) ligand 2* (Burgess et al. 2010). In addition, the influence of the pro-inflammatory transcription factors nuclear factor kappa B and activating protein-1 in the early host pro-inflammatory response to *P. ovis* has been identified (Burgess et al. 2011).

A major feature of sheep scab is the rapid epidermal influx of eosinophils and neutrophils, followed by blister formation and a serous fluid exudate resulting in dermal oedema (van den Broek et al. 2004). The sheep scab lesion is histopathologically consistent with those described for allergic dermatitis (Stromberg and Fisher 1986). IgE-mediated immediate hypersensitivity responses might participate in the lesion pathology or immunity during the later stages of infestation; however, the initial process of establishment involving pro-inflammatory responses is unlikely to involve IgE-mediated reactions, as this occurs in mite-naive sheep. The mechanism of this early pro-inflammatory response is unknown but represents a pivotal step in mite colonisation and is critical in determining disease progression. Prior infestation alters the progression of subsequent

infestations and reductions in lesion size have been observed in secondary infestations in sheep, suggesting that vaccination against the parasite might be feasible (Bates 2000). Vaccination with *P. ovis* whole-mite extracts has resulted in a 15-fold reduction in mite numbers and a four-fold reduction in lesion size (Smith et al. 2001, 2002). However, identification of the individual proteins involved has proved challenging.

Scabies mites

Scabies was one of the first infectious diseases for which the aetiology was determined when Giovani Cosimo Bonomo described the mite *S. scabiei* as the causative agent of the disease in 1687 (Ramos-e-Silva 1998). Despite this early recognition of the cause, scabies remains a poorly understood and neglected disease that adversely affects the quality of life of millions of people (Jin-gang et al. 2010). The World Health Organisation classifies scabies among the six epidermal parasitic skin diseases that are of particularly high prevalence and intensity of infection within populations of the world living in poverty (Feldmeier and Heukelbach 2009). Annually, an estimated 300 million people face physical, mental, social and economic stigma as a result of this illness (Taplin et al. 1990). Scabies also infects millions of other animals of more than 40 known mammalian species throughout the world (Fain 1978).

Scabies mites are obligate parasites with a life span of around 30–60 days. Female scabies mites increase in size after mating and burrow into the upper epidermis of the host at a rate of 0.5–5 mm/day. They lay their eggs within the burrows and these eggs hatch into larvae within about 3 days. The larvae transform to protonymphs then to tritonymphs and subsequently to adult male and female mites, with the whole life cycle spanning about 14 days (Alexander 1984). All life stages from larvae onwards are thought to leave their burrows and traverse the surface of the host skin and are potentially infective if transmitted to further hosts.

Clinical features and transmission

In humans, two major forms of the disease are recognised: ordinary scabies and crusted scabies. Ordinary scabies commonly manifests in the form of cutaneous inflammation, pruritis and skin lesions (Mellanby 1943). Common sites of infestation are the web of the fingers, the volar aspects of the wrists, the extensor aspect of the elbows, the pelvic girdle including the buttocks, the peri-areolar region in females and genitalia in males (Walton et al. 2004b; Hengge et al. 2006). Skin irritation can be localised at these sites or comprise a more generalised secondary sensitisation and is reported to be more intense at night. The number of mites is highest at the beginning of an ordinary scabies infestation but is usually self-limiting and drops down to approximately 10–12/g skin. In a first infestation, symptoms usually occur 4–6 weeks after initial infestation (Mellanby 1944) consistent with a type-IV delayed hypersensitivity reaction, namely a cell-mediated antibody-independent immune response (Mellanby 1944; Dahl 1983; Cabrera and Dahl 1993; Hengge et al. 2006; Elder et al. 2009). The symptoms result from immune responses to mite products; saliva, eggs and faecal pellets (Hengge et al. 2006). However, with subsequent infestations, the symptoms usually reappear in 24–48 h. This is attributable to the immune response produced by the host in response to scabies mite antigens, a response that can remain positive for up to a year (Falk 1980).

Crusted scabies, also known as Norwegian scabies, is a rare form of the disease that affects a small subset of individuals. Crusted scabies often presents as a psoriasiform dermatitis with prominent hyperkeratosis (Hengge et al. 2006). It can be extensively distributed over the body including regions not usually affected in ordinary scabies, such as the neck, scalp and face (Walton et al. 2004b). Severe crusting and fissuring of the skin is common and can result in serious secondary bacterial infections. In this form of the disease, the mite burden is extremely high and is reported to be as many as 4700 mites per gram of skin (Currie et al. 1995) rendering affected individuals highly contagious. Outbreaks of disease from index cases of crusted scabies result in ordinary scabies in subsequent cases (Moberg et al. 1984; Holness et al. 1992; Estes and Estes 1993), supporting genetic analyses that indicate that the same variety of mites is responsible for both forms of the disease (Walton et al. 1999a, b). Crusted scabies has been reported in individuals with cognitive (Kolar and Rapini 1991) and immunological (for a review, see Walton et al. 2004b) deficits; however, the disease is also seen in immune-competent individuals for whom any underlying susceptibility is unknown (Gogna et al. 1985; Currie et al. 1995). Clustering within families suggests a genetic predisposition to this form of the disease (Roberts et al. 2005).

The transmission of scabies is predominantly via direct skin contact and, as such, it prevails in communities with overcrowded living conditions. Survival of the mites away from the host depends on the ambient conditions of temperature and humidity, with highest survival rates in environments of low temperature and high relative humidity. Scabies mites are extremely prone to desiccation and transmission via fomites is thought to be rare (Mellanby 1941).

Interaction with the host epidermis

The burrows of scabies mites are commonly found in locations in which few hair follicles are present and the stratum corneum is soft and thin (Alexander 1984). In ordinary scabies, the mites are most widely believed to reside in the

stratum corneum with their gnathosoma embedded into deeper layers of the epidermis, either the stratum granulosum (Christopherson 1986) or deeper into the stratum mucosum (Alexander 1984). Scabies mites have a feeding mechanism that consumes large amounts of salivary secretions while feeding on the host skin (Arlian et al. 1984). They have also been shown to lose their body water at a rate that is the highest among astigmatid mites. Arlian et al. (1988) have argued that the stratum corneum is too dry to provide sufficient moisture and thus the only possible mechanism for the mites to replenish lost body water is from deeper epidermal layers (Arlian 1988). Furthermore, the burrows of scabies mites have been reported to contain an amorphous material that is believed to originate from the epidermal layers below the stratum corneum (Van Neste 1984). The most convincing evidence for the access of mites to deeper skin layers comes from crusted scabies patients in whom most of the mites reside in the moist stratum granulosum, stratum basale or even as deep as the dermoepidermal border (Van Neste and Lachapelle 1981; Arlian 1988). The importance of serous material for mite nutrition has been demonstrated in an in vitro culture system in which mites have been shown to survive longer on media that contain serum (Arlian 1988). Insights into the feeding behaviour of scabies mites have also been obtained from a study showing the absence of any lipase activity in scabies mite extracts; presumably, scabies mites cannot survive on the lipophilic secretions of the skin and would prefer to reside as close to the dermis as possible to fulfil their nutritional requirements (Morgan and Arlian 2006). Recent work has confirmed the presence of scabies mites in the stratum granulosum by using reflectance confocal microscopy (Levi et al. 2011). Moreover, immunohistological data have demonstrated the localisation of ingested host filaggrin (Beckham et al. 2009) and complement components C1q (Bergstrom et al. 2009) and C9 (Mika et al. 2011) in the digestive tract of scabies mites infesting human skin.

Histological studies have reported that infestation with scabies mites results in a number of pathological changes in both the epidermal and dermal layers of the skin (Hejazi and Mehregan 1975; Hoefling and Schroeter 1980). The histopathological features of scabies lesions are similar to those of a dermal hypersensitivity response. The permeability of the dermal and epidermal vesiculation is increased leading to the migration of lymphocytes and eosinophils. IgM and fibrin are deposited in the dermoepidermal junction and complement C3 and IgG are deposited in the mite burrows and on the surface of the mites. An increase in vesicular permeability also leads to the non-specific deposition of serum proteins in vessel walls in the dermoepidermal junction and even the stratum corneum (Hoefling and Schroeter 1980). Scabies commonly gives rise to severe itching and puritis. Scratching by the host often leads to regions of haemorrhagic crusting and excoriations (Hejazi and Mehregan 1975).

Limited data currently exist to advance the understanding of the innate and adaptive host immune response in scabies. Roberts et al. (2005) have reported markedly altered immune functions in crusted scabies patients. In particular, low systemic serum levels of the complement components C3 and C4 have been seen in crusted scabies (Roberts et al. 2005), whereas levels in ordinary scabies lie within the normal range (Falk 1980; Hoefling and Schroeter 1980; Falk and Eide 1981; Salo et al. 1982). Increased deposition of C3 into the dermal blood vessels of both crusted and ordinary scabies patients (Hoefling and Schroeter 1980; Walton et al. 2008) and the localisation of C1q (Bergstrom et al. 2009) and C9 (Mika et al. 2011) to the mite gut suggest increased local activation of the complement system. Given the large number of mites and mite products in crusted scabies, elevated levels of local complement deposition would be expected in this inflammatory environment. This correlates with the presence of the complement-inhibiting scabies mite inactivated protease paralogues (SMIPP) in the mite burrow and the mite gut (Willis et al. 2006).

In crusted scabies, skin lesion biopsies contain eosinophils in the dermis and blood, lymphocytes and elevated levels of IgE (Walton et al. 2008). In vitro studies have found that whole-mite extract modulates the regulation of inflammatory cytokines in keratinocytes, fibroblasts, human peripheral blood mononuclear cells and dendritic cells (Arlian et al. 1996, 2003; Elder et al. 2006, 2009; Mullins et al. 2009). More recently, *S. scabiei* recombinant proteins have been used to investigate immune responses (Harumal et al. 2003; Dougall et al. 2005; Walton 2010). These studies indicate that crusted and ordinary scabies patients display an IgE and IgG response to specific antigens, with IgE responses being elevated in crusted scabies patients. Complementary immunohistological studies also show that both IgG (Rapp et al. 2006) and IgE (Walton 2010) localise to the mite gut and the burrow. The peripheral blood mononuclear cells of ordinary scabies patients have been shown to produce higher levels of the cytokines interferon-γ and IL-4. In contrast, the peripheral blood mononuclear cells of crusted scabies patients preferentially produce IL-5 and IL-13 (Walton 2010).

Keratinocytes and dendritic cells are in intimate contact with scabies mites. In crusted scabies, the mites appear to interfere with desquamation, causing hyperkeratosis and preventing loss of eggs and hatching larvae. The striking epidermal thickening in crusted scabies might result from interference with the desquamation pathway by secreted/excreted intestinal mite proteins. An understanding of such interactions with the host epidermis would be a first step towards therapeutic interference.

Identification of mite allergens

Chapman et al. (2007a) have recently provided a detailed review of the current status of allergen nomenclature, protein families and biological functions of allergens from mites, animal dander, pollens, insects and foods. The system is simple, involving the use of abbreviated Linnean genus and species names followed by an Arabic number to indicate the chronology of allergen purification. Allergens are found in more than 120 distinct protein families, among them being many proteases and some protease inhibitors (Chapman et al. 2007a). Strictly speaking, an allergen is defined as a non-parasitic antigen capable of stimulating a type-I hypersensitivity reaction in atopic individuals (Goldsby et al. 2000). In house dust mites, allergens are commonly defined as molecules to which IgE binding has been identified in at least 5 % of house dust mite allergic individuals (Thomas et al. 2007). Homologues of house dust mite allergens are apparent in many parasitic mite species.

House dust mites

The allergens of house dust mites have been extensively studied because of their role as major contributors to respiratory disease in humans (Thomas et al. 2002). These allergens include proteases, which are localised to the midgut and hindgut of the mites, indicating a role in digestive processes (Chua et al. 1988; Stewart et al. 1992a, 1994; Smith et al. 1994; Thomas and Smith 1999). Currently, over 20 identified house dust mite allergen groups have been defined.

Approximately half the IgE in house dust mite allergy is directed against two major allergens: the group 1 (cysteine protease) and group 2 (MD-2-related lipid-recognition domain lipid-binding protein) allergens. Much of the remainder of the IgE response is directed against four further allergens: group 4 (α-amylase), group 5 (unknown identity), group 7 (lipid-binding protein) and group 21 (unknown identity but with homology to the group 5 allergen; Hales et al. 2006; Weghofer et al. 2008a, b). This hierarchy is consistent in patients allergic to house dust mites and is not a function of their total IgE levels or the severity of disease (Hales et al. 2006, 2009). The allergenicity of some of the molecules is thought to be exacerbated by their protelytic activity, which activates inflammatory processes.

D. pteronyssinus and *D. farinae* allergens typically show 80 %–85 % amino-acid-sequence identity to each other. Whereas cross reactivity between some allergens has been demonstrated, each species also has unique epitopes (Thomas et al. 2007). The glycyphagid mite *Blomia tropicalis* generally shows 30 %–40 % amino-acid-sequence identity to homologous allergens in *D. pteronyssinus* and *D. farinae* (Chua et al. 2007). Cross reactivity between allergen extracts has been shown (Chew et al. 1999a); however, the major *B. tropicalis* allergens, namely Blo t 5 and Blo t 21, do not cross react with *Dermatophagoides sp.* by IgE binding (Arruda et al. 1997) or skin test (Simpson et al. 2003).

Sheep scab mites

Initial studies of whole-mite extract identified a range of protease activities in *P. ovis* and the rabbit mite *Psoroptes cunculi* including phosphatase, esterase, cysteine protease and aspartic protease activity (Nisbet and Billingsley 1999, 2000, 2002). In order to identify potential vaccine targets against sheep scab, serum from successful vaccine trials was used to immunoscreen a cDNA library constructed from mixed-stage and gender *P. ovis* mites. Immunodominant mite factors recognised by host IgG included a catchin-like protein, a novel mu class glutathione S-transferase, tropomyosin and paramyosin (Nisbet et al. 2006). Additional mite allergens have also been identified, a number of which represent homologues of the major house dust mite allergens, e.g. Der p 1 (termed Pso o 1), Der p 2 (Pso o 2), Der p 3 (Pso o 3), Der p 5, Der p 7, Der p 8, Der p 10, Der p 11 and Der p 13 (Lee et al. 1999, 2002; Temeyer et al. 2002; Kenyon et al. 2003; McNair et al. 2010). In a study by Kenyon et al. (2003), the most abundant allergen was found to be the group 2 homologue Pso o 2, which constituted approximately 4 % of all transcripts. Analysis of transcripts differentially expressed between fed and starved *P. ovis* mites identified an association between feeding activity and increased expression of Pso o 1, 5, 7 and 13 (McNair et al. 2010). The majority of the *P. ovis* expressed sequence tag (EST) sequences (961 ESTs, 62 %) and showed similarity to known genes allowing functional analysis. Amongst these, further homologues of house dust mite and tick salivary factors were identified, offering new insights into *P. ovis* biology.

Scabies mites

Antisera from scabies patients have been shown to contain antibodies that recognise *D. pteronyssinus* antigens indicating the presence of similar antigens (Falk and Bolle 1980; Falk et al. 1981; Arlian et al. 1988, 1995; Morgan et al. 1997). A study of protease activity in a range of astigmatid mites has failed to detect trypsin and chymotrypsin activity in whole scabies mite extract (Morgan and Arlian 2006). However, this could be attributable to the low sensitivity of the colorimetric assay utilised, which also failed to detect chymotrypsin activity in house dust mite extracts, although the faecal matter of these organisms is known to contain chymotrypsin activity (Stewart et al. 1994). The presence of aspartic protease and cysteine protease activity in *S. scabiei* extract has subsequently been demonstrated (unpublished data).

Molecular analysis of scabies mites has lagged behind that of house dust mites, because of the lack of an in vitro

culture system for the propagation of mites resulting in a reliance on mites collected from infested humans or other animals. The first potential scabies allergens were identified by the immunoscreening of *S. scabiei* expression libraries. This approach initially identified clones encoding paramyosin and myosin from fox scabies mites (Mattsson et al. 2001) and a fragment of a molecule with homology to the house dust mite group 14 allergen from human scabies mites (Harumal et al. 2003). Scabies research was significantly advanced by two EST analyses that undertook the sequencing of cDNA clones from fox mites (*S. scabiei* var. *vulpes*; Ljunggren et al. 2003) and human mites (*S. scabiei* var. *hominis*; Fischer et al. 2003a, b). The sequencing of 1020 clones of the fox mite library identified 652 different sequences that included homologues of the house dust mites group 1 (cysteine protease), group 8 (glutathione S-transferase), group 10 (tropomyosin) and group 13 (fatty-acid-binding protein) allergens (Ljunggren et al. 2003). Over 43,000 cDNA clones were sequenced from the human mite library, with normalisation of the cDNA libraries being undertaken after approximately 10,000 sequences, to improve the representativeness of the ESTs (Fischer et al. 2003a, b). This resulted in the identification of many further homologues of the house dust mite allergens (Fischer et al. 2003a; Holt et al. 2003, 2004; Dougall et al. 2005) and of molecules implicated in drug resistance in other organisms (Mounsey et al. 2006, 2007; Pasay et al. 2006, 2008), molecules with immunodiagnostic potential (Fischer et al. 2003a; Walton et al. 2010) and sequences useful for examining population structure (Walton et al. 2004a). Several scabies mite protein families have subsequently been characterised and functionally defined as having crucial roles in the host-parasite interaction (see below).

House dust mite intestinal proteases and their homologues in the parasitic mites *P. ovis* and *S. scabiei*

Homologues of many of the house dust mite allergens have been identified in the parasitic mites *P. ovis* and *S. scabiei*, as indicated above. Many of these are active enzymes for which their biochemical function might enhance their allergenicity. Parasitic proteases facilitate the invasion and digestion of host tissue, mediate moulting and help parasites evade the host immune response; they are therefore considered to be potential targets for the development of novel immunotherapeutic, chemotherapeutic and serodiagnostic agents. Of particular interest are the mite intestinal proteases, the most important of which are the cysteine, serine and aspartic proteases.

Group 1 cysteine proteases

Cysteine proteases play an important role in many parasitic organisms including essential catabolic functions, immunoevasion, invasion or destruction of tissues and cells and encystment, excystment and exsheathing (Sajid and McKerrow 2002). The group 1 allergens are members of the papain-like cysteine proteases and are a major house dust mite allergen predominantly found in mite faecal pellets (Yasuhara et al. 2001a, b). Der p 1 was the first allergen to be cloned and sequenced (Chua et al. 1988). Its sequence information suggested structural homology to cysteine proteases such as papain and actinidin, as was later confirmed in X-ray crystal structures of recombinant forms of the pro-enzyme (Meno et al. 2005) and the mature active protease (de Halleux et al. 2006) of Der p 1. Der p 1 is a magnesium–binding protease with a dimeric structure, possibly explaining its stability and persistence in the environment. Der p 1 and Der f 1 have been produced as secreted, non-glycosylated and enzymatically active recombinant allergens with pure cysteine protease activity (Best et al. 2000; van Oort et al. 2002; Takai et al. 2005). The translated amino acid sequence of Eur m 1 from *E. maynei* displays over 80 % sequence identity with the corresponding allergens from *D. pteronyssinus* and *D. farinae* (Smith et al. 1999) and hence the group 1 allergens from the three house dust mite species most likely have identical functional properties. A series of studies (for a review, see Chapman et al. 2007b) has provided accumulating evidence of the allergenic activity of the group 1 allergens: (1) they promote IgE synthesis by cleaving CD23 and CD25 from activated B and T cells, respectively (Hewitt et al. 1995; Schulz et al. 1995, 1998; Shakib et al. 1998); (2) they damage lung epithelia (King et al. 1998; Tomee et al. 1998); (3) they degrade intracellular tight junctions by cleaving at Der-p-1-specific cleavage sites in tight junction membrane proteins of bronchial epithelial cells (Wan et al. 1999); and (4) this damage is associated with the release of proinflammatory cytokines from epithelial cells and infiltrating mast cells and basophils (King et al. 1998; Tomee et al. 1998). Further studies have shown that cytokine release from epithelial cells is mediated by the activation of protease-activated receptor-2 (PAR-2; Asokananthan et al. 2002).

Previous EST analysis of *P. ovis* has identified three distinct cysteine proteases encoding for two putative cathepsin-B and one cathepsin-L-like protein, termed Pso CathB1, Pso CathB2 and Pso CathL1, respectively (Kenyon and Knox 2002). In addition, a cDNA clone (*Pso o 1*) encoding for a *P. ovis Der p 1*-like cysteine proteinase homologue has been isolated (Lee et al. 2002). Recombinant Pso o 1 has been assessed for IgG and IgE recognition in the serum from *P. ovis*-naive and *P. ovis*-infested sheep; the authors have demonstrated that Pso o 1 is indeed immunogenic, with Pso-o-1-specific IgG and IgE being detected in post-infestation serum (Lee et al. 2002). Further studies by Nisbet and colleagues (Nisbet et al. 2007; McNair et al. 2010) have shown that antibodies generated against recombinant Pso o 1 cross-react with Der p 1 and that Pso o 1 is

also up-regulated in fed vs. starved *P. ovis* mites indicating a potential role in mite digestive processes.

Although only a single group 1 cysteine protease allergen has been identified in house dust mites and in *P. ovis*, the ESTs of the burrowing mite *S. scabiei* var. *hominis* contain five cysteine proteases with homology to the group 1 house dust mite allergens, termed Sar s 1 a–e. Similarly, Ljunggren et al. (2003) have reported five cysteine protease sequences with homology to Der f 1 and Eur m 1 in *S. scabiei* var. *vulpes* ESTs. Surprisingly, in addition to these, a further five contigs also show homology to group 1 allergens; however because of a mutation of at least one of the residues of the catalytic site, they are predicted to be inactive as proteases. These molecules have been termed SMIPP-cysteine proteases (SMIPP-Cs; Holt et al. 2004). Expression of one proteolytically active and one proteolytically inactive member of this family has recently been accomplished in *Pichia pastoris* and functional analysis is underway (unpublished data).

Group 3 allergens

The group 3 house dust mite allergens, first described by Heymann et al. (1989), are serine proteases related to trypsin and chymotrypsin (Stewart et al. 1989, 1992b; Rawlings and Barrett 1994). These proteases are a major constituent of mite faeces, despite their paucity in mite body extracts (Stewart et al. 1994). Reports of the frequency and intensity of IgE binding of the group 3 house dust mite allergens vary considerably (Heymann et al. 1989; Ando et al. 1993; Yasueda et al. 1993; King et al. 1996; Hales et al. 2006; Kidon et al. 2011), possibly because of differences in the purity of the protein analysed or the presence of sequence variants (Thomas et al. 2002). Functional studies of Der p 3 have demonstrated that this enzyme and Der p 1 and Der p 9, can stimulate PAR-2 expressed by lung epithelial cells (Sun et al. 2001), thereby mediating, at least in part, the release of proinflammatory cytokines and the triggering of an inflammatory response.

Previous analysis of the skin response to *P. ovis* infestation has demonstrated the down-regulation of a number of genes involved in the process of epidermal differentiation, i.e. filaggrin, loricrin and involucrin (Burgess et al. 2010). One of the main implications of the down-regulation of genes involved in the maintenance of an effective skin barrier in sheep scab pathogenesis could be the initiation of a cycle of barrier disruption, followed by increased allergen penetration leading to a further reduction in barrier integrity.

In contrast to the single-copy group 3 allergens in dust mites and *P. ovis*, a multicopy S1-like family of serine proteases (related to chymotrypsin and trypsin) has been identified in *S. scabiei*, with only one of the 33 members being predicted to be catalytically active (Holt et al. 2003; Fischer et al. 2009). The single active serine protease (designated Sar s 3) resembles the group 3 allergens in having six cysteine residues involved in three disulphide bonds, an intact catalytic triad with histidine at position 46, aspartic acid at position 93, serine at position 202 and a signal peptide. Functional studies of Sar s 3 have confirmed that it is an active serine protease with trypsin-like activity. Silico analysis has predicted that the protease would have a preference for proteases with RSG or RSA sites in the P1-P2' position and has generated a list of prospective targets. Among the top 100 predicted human substrates is a range of skin proteins. The skin protein filaggrin, which is a key component of the stratum corneum and contains numerous RSG sites, has been tested and found to be effectively digested by Sar s 3. Sar s 3 and filaggrin have been co-localised to the scabies mite digestive system (Beckham et al. 2009).

Each of the other 32 distinct contigs has been identified as a serine protease with closest BLASTx matches to the group 3 house dust mite allergens Der p 3, Der f 3 and Eur m 3. Apart from Sar s 3, all members of this family identified to date have a mutation in at least one residue of the active site triad (Holt et al. 2003) and lack one of three pairs of cysteine residues. Such dramatic changes in the catalytic site indicate that these proteins cannot function as active serine proteases by any known mechanism. Hence, they have been termed SMIPP-serine proteases (SMIPP-Ss). Limited investigation of individual mites has suggested the expression of Sar s 3 and of more than one SMIPP-S in adult mites (Holt et al. 2003) and all larval stages except eggs (unpublished data).

These inactivated serine proteases (SMIPP-Ss) have subsequently been shown to be used by scabies mites as a means of evasion from the host innate immune response (Bergstrom et al. 2009). This study has identified two SMIPP-Ss, selected from different clades in the phylogenetic tree of this family, as potent inhibitors of the complement system (Bergstrom et al. 2009). Extensive functional complement assays, followed by complement deposition and binding assays, have led to the conclusion that both SMIPP-Ss bind to the complement factors C1q, mannose-binding lectin and properdin. These three complement molecules are essential for the activation of the three pathways of the complement system. Interference with these three major complement components might enable the mites to evade host immune defences. To assess the effect of additional SMIPP-Ss on complement, further micro-titer plate-based deposition assays have been performed. All SMIPP-Ss tested to date also prevent the activation of all complement pathways in these enzyme-linked immunosorbent-based functional assays (manuscript under review). The biological relevance of these findings has been reinforced

by the localisation of the human complement components C1q and C9 to the gut of scabies mites in correlation with the absence of the membrane attack complex in the mite gut (Mika et al. 2011). This indicates that the anti-complement machinery could indeed be highly efficient in vivo. Furthermore, the inactivation of complement by these SMIPP-Ss might aid in the efficient growth of group A *Streptococcus* in the microenvironment of the burrows. If this molecular link between scabies and bacterial infections can be determined, it may provide new avenues for developing alternative treatment options against this neglected disease.

Complementary to the functional investigations, structural studies of the same two SMIPP-Ss have identified the mechanism behind the inactivation of these proteases (Fischer et al. 2009). The crystal structures of two SMIPP-Ss have been determined at resolutions of 1.85 Å and 2.0 Å and have been found to adopt the general fold of serine proteases, except for one major structural variation. Compared with the active serine protease Sar s 3, all SMIPP-Ss discovered to date lack a third disulphide bond. This enforces a structural rearrangement that results in the binding pocket being blocked by a large residue (typically a tyrosine at amino acid position 200) and, without further structural rearrangements, the binding pocket probably cannot facilitate protein-protein interaction. To test peptide substrates for binding, three SMIPP-Ss were screened against a 20mer phage display library and no binding was observed. The cumulative effects of a mutated catalytic triad, a blocked binding pocket and an inability to bind protein or peptides at the canonical binding site is concluded to render the proteases proteolytically inactive and unable to function as canonical serine proteases. It has been proposed that the SMIPP-Ss have evolved non-catalytic binding activities and that an exosite may be used as an alternative binding site. Analysis of the predicted molecular surface properties of all 32 SMIPP-Ss has identified regions of conservation opposite the binding pocket on the other side of the molecule; these regions could present alternative sites for interaction (Fischer et al. 2009).

Aspartic protease

Aspartic proteases are a class of endopeptidases with a pair of aspartic acid residues at their active site. The A1 family is the largest and most widely studied class of aspartic proteases and includes pepsin, renin and cathepsin D. Aspartic proteases are known to mediate a range of diverse and essential physiological functions such as tissue penetration, migration and digestion. In addition, many blood-feeding parasitic organisms are thought to utilise aspartic proteases as part of a haemoglobin digestion cascade. The malaria parasite *Plasmodium falciparum* (Banerjee et al. 2002) and the human and canine hookworms *Necator americanus* and *Ancylostoma caninum* (Williamson et al. 2002) are known to use aspartic proteases for the digestion of haemoglobin.

Haemoglobin-, fibrinogen- and fibronectin-degrading activity has been identified in house dust mite extract (Morgan and Arlian 2006); however, an aspartic protease is not among the house dust mite allergens identified to date. Haemoglobin-digesting activity has been detected in whole *Psoroptes* mite extract, with optimum activity seen at pH 3.5. This acid proteinase activity is inhibited by the aspartic protease inhibitor pepstatin A and is higher in mites raised on rabbits (*P. cuniculi*) than on sheep (*P. ovis*; Nisbet and Billingsley 1999), a finding that is consistent with differences in diet for these conspecific mites when raised on different hosts, with the mites feeding on whole blood on rabbits compared with serous exudates on sheep. A cathepsin D-like enzyme has subsequently been characterised and its presence in the soluble fraction of whole-mite extract indicates that it is likely to be cytosolic or luminal in nature (Nisbet and Billingsley 2002).

Haemoglobin-digesting activity at acidic pH has also been detected in whole scabies mite extract (unpublished data). A cathepsin-D-like aspartic protease sequence has been identified in ESTs from the fox scabies mite (Ljunggren et al. 2003) and from the human scabies mite, with the latter subsequently being expressed as an active recombinant enzyme (unpublished data). Anti-sera raised against the protein in mice have localised the protein to the mite gnathosoma, midgut, hind gut, faecal pellets and eggs. This is consistent with the pattern of expression that occurs throughout the mite lifecycle, with the greatest expression in adult female mites. The recombinant enzyme has been shown to digest human haemoglobin, serum albumin, fibrinogen and fibronectin; however, no digestion of collagen III or laminin has been detected (unpublished data).

Studies in hookworms have revealed that despite having active-site clefts with identical primary sequences, the aspartic proteases of the human hookworm *Necator americanus* and the dog hookworm *Ancylostoma caninum* have different substrate preferences. Each aspartic protease is capable of digesting haemoglobin, some skin macromolecules and serum proteins from their permissive host with greater efficiency than their non-permissive host, thus contributing to their host specificity (Williamson et al. 2003). Scabies mites also show evidence of host specificity, with dog mites unable to establish permanent infestation on humans (for a review, see Walton et al. 2004b). Reasons for this host specificity have been proposed to include physiological differences in dietary requirements (Arlian 1989). The aspartic protease from dog and human scabies mites has been shown to have sequence differences (unpublished data) and work is underway to investigate any role of these proteases in the host specificity of scabies mites.

Aspartic proteases are the focus of inhibitor drug design for a range of pathogenic organisms including viruses, fungi and protozoa. Aspartic protease inhibitors have been successfully used to treat HIV infection and hypertension in humans. The molecular specificity of parasite proteases indicates the strong potential of inhibitor therapies that specifically target parasite enzymes (Williamson et al. 2003). Indeed, the inhibition of aspartic protease activity with pepstatin A in hookworms decreases larval migration through skin, demonstrating that inactivation of just one enzyme can significantly impair or completely disrupt the ability of the parasite to feed (Brown et al. 1999; Williamson et al. 2003).

Concluding remarks

Mite proteases undoubtedly play key roles in triggering and modulating host tissue responses. Proteolytic dust mite allergens (e.g. Der p 1, Der p 3, Der p 6 and Der p 9) can cleave the low-affinity IgE receptor, can promote Th2 responses and have proinflammatory effects by initiating the release of Th2 cytokines. *P. ovis* is distinct in that it appears to rely upon the proteolytic and allergenic actions of its enzymes to generate a food source (i.e. serous exudate induced by the host pro-inflammatory response) and also to provide a suitable microclimate (i.e. a combination of heat and high humidity) for its own survival. Scabies mites, however, have more complex interactions with their host. When invading the skin, the mite is not merely dealing with dead skin but with live epidermal protein, the host immune system and other complex machineries, such as coagulation cascades and the desquamation pathway. In addition to digestion, the mite must also combat any host molecules that might threaten digestive activity within its gut. Consequently, the role of intestinal proteases has diversified and certain protease species that are single copy genes in the house dust mites and sheep scab mite have expanded into multicopy families in scabies mite. Some functions of these have been described; however, given their high copy numbers and diversity, further effects on other host pathways should not be excluded. The identified inactivated protease families of the scabies mite might block, for example, the activation of PAR-2 on the surface of keratinocytes. Such inhibitors could have important implications for the development of novel asthma therapeutics, as activation of PAR-2 receptors in the lungs is known to play a role in the pathogenic process in house dust mite allergy. Despite their allergenic side effects after excretion, the biological function of active proteases in the mite is presumably their role as digestive proteases and, as such, they might be amenable to therapeutic inhibition.

A lack of available genome sequence data is hampering further discovery of novel therapeutic and vaccine candidate molecules for these mite species. One advance for mite genomics has been the publication of a preliminary genome survey for the honey bee mite *Varroa destructor* (Cornman et al. 2010) and, recently, the genome sequence of the two-spotted spider mite *Tetranychus urticae* (Grbic et al. 2011). Only the mitochondrial genome sequence is available for *D. pteronyssinus* (Dermauw et al. 2009) and no full genomic DNA sequence is available for *D. pteronyssinus*, *P. ovis* or *S. scabiei*. The main focus to date has been on the generation and analysis of ESTs (Lee et al. 2002; Fischer et al. 2003a, b; Kenyon et al. 2003; Ljunggren et al. 2003; Angus et al. 2004), which offer insights into mite biology and host interactions. Comparative analysis of the ESTs of the free-living *D. pteronyssinus* with the parasitic *P. ovis* and *S. scabiei* might highlight further key mechanisms by which these mites induce specific responses or interact with their host. Advances in sequencing technology are currently transforming our ability to generate de novo genome sequence data. The recent determination of the genome sizes of these three important mites (Mounsey et al. 2012) indicates that full genome sequences are achievable. Comparative genomic analyses should provide significant advances in our understanding of these important mite species and the diseases that they cause.

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