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Expressed sequence tag survey of gene expression in the scab mite *Psoroptes ovis* – allergens, proteases and free-radical scavengers

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SUMMARY

Psoroptes ovis, the causative agent of sheep scab, is an important ectoparasitic mite infecting sheep, goats and cattle. Infection is characterized by an extensive dermatitis, scab formation and intense itching. Initial focal lesions spread outwards, coalesce and may extend over the whole body. The host response to infestation has all the characteristics of an immediate-type hypersensitivity reaction but the mite antigens and allergens which initiate this response are almost completely undefined. Here, 507 randomly selected cDNAs derived from a mixed population of *P. ovis* were sequenced and the resultant nucleotide sequences subjected to Cluster analysis and Blast searches. This analysis yielded 280 clusters of which 49 had >1 sequence with 24 showing significant Blast X homology to another protein in the databases. There were 231 sequences which appeared on one occasion and 109 of these showed significant Blast X homology to other sequences in the databases. This analysis identified homologues of 9 different types of allergens which have been characterized in other allergic conditions such as responses to house dust mites. It also identified a number of cysteine proteases which may contribute to lesion development as well as several free-radical scavenging enzymes which may protect the mite from host immune effector responses.

Key words: sheep scab, *Psoroptes ovis*, expressed sequence tag, allergens.

INTRODUCTION

Psoroptes ovis, the causative agent of sheep scab, is an important ectoparasitic mite infecting sheep, goats and cattle. The condition is particularly prevalent in sheep in the UK and cattle in Europe and the USA and is highly contagious being spread by direct or indirect contact. Infection is characterized by an extensive dermatitis, scab formation and intense itching and infected animals show pronounced behaviour responses including rubbing the area of the scab lesion, scratching, wool loss, wounding and fitting. Initial focal lesions spread outwards, coalesce and may extend over the whole body.

The host response to infestation has all the characteristics of an immediate-type hypersensitivity reaction and this response can be experimentally induced by injection of mite extracts intradermally (van den Broek *et al*. 2000). To date, the individual antigens which invoke the characteristic response in sheep scab remain largely undefined. Immunoblots of mite extracts have been probed with sera from immune or infested sheep. Between 6 and 30 antigens were detected but their nature was undefined (e.g. Boyce & Brown, 1991). Another study identified a 16 kDa allergen which is a mite group 2 allergen homologue (Pruett, 1999; Temeyer, Soileau & Pruett, 2002). Biochemical analyses have shown that the mites contain cysteine, metallo- and aspartyl proteinases (Nisbet & Billingsley, 1999, 2000; Kenyon & Knox, 2002). These enzymes can degrade proteins such as collagen, fibronectin, haemoglobin and fibrinogen, suggesting a role in mite feeding and lesion formation (Kenyon & Knox, 2002).

Allergens have been extensively studied in the house dust mites *Dermatophagoides pteronyssinus*, *D. farinae*, *D. microceras* and *Euroglyphus maynei* with comparative data from storage mites such as *Lepidoglyphus destructor* and the tropical mite *Blomia tropicalis* (Stewart, 1995). Each species contains several allergens with considerable interspecies similarities. The allergens are often proteases (peptidases) such as cysteine proteases (e.g. Der p 1; Chua *et al*. 1988) and serine proteases (Stewart *et al*. 1992). Other peptidases identified include amino- and carboxypeptidases (Stewart *et al*. 1992) but their allergenicity remains to be confirmed (Stewart, 1995). These enzymes are commonly found in mite faecal pellets and may facilitate both intracellular

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Table 1. Summary of the EST dataset showing those ESTs encoding proteins with similarity to allergens, proteases, free-radical scavenging enzymes and others of general interest

(For the clusters comprising more than 1 EST, the accession number of the first EST in the cluster is shown. All ESTs can be viewed at http://nema.cap.ed.ac.uk/Chelicerates/ChelDB.php.)

Description and Accession no.	No. of ESTs	Gene name	Putative function	Homologue in	Blast X E-value	ORF length (aa)	Homology (%)	
							Identity	Similarity
Allergens								
BQ834604	20	*Pso-o-II*	Group II allergen	*P. ovis*	$1{\cdot}2\ e^{-72}$	160	99	99
BQ834615	9	*Pso-derF A*-1	Secreted cysteine protease	*Dermatophagoides farinae*	$2{\cdot}1\ e^{-73}$	231	69	79
BQ834608	4	*Pso-gst*-2	Glutathione S-transferase	*Drosophila melanogaster*	$9{\cdot}8\ e^{-21}$	141	45	65
BQ834621	4	*Pso-fabp*-1	Fatty acid binding protein	*Blomia tropicalis*	$8{\cdot}8\ e^{-20}$	134	35	53
BQ834778	3	*Pso-gp7*-1	Unknown, group 7 allergen	*Lepidoglyphus destructor*	$1{\cdot}2\ e^{-22}$	178	35	57
BQ834975	2	*Pso-der p V*-1	*Der p* V IgE binding allergen	*D. pteronyssinus*	$8{\cdot}0\ e^{-35}$	154	40	64
BQ834874	1	*Pso-tropo*-1	Tropomyosin	*D. farinae*	$7{\cdot}6\ e^{-94}$	212	98	98
BQ834786	1	*Pso-paramy*-1	Paramyosin	*Sarcoptes scabiei*	$1{\cdot}5\ e^{-58}$	172	90	93
BQ835043	1	*Pso-hsp*-1	Heat shock protein	*Caenorhabditis elegans*	$9{\cdot}2\ e^{-13}$	149	41	58
Cysteine proteases								
BQ834615	9	*Pso-der F* A-1	Secreted cysteine protease	*D. farinae*	$2{\cdot}1\ e^{-73}$	231	69	79
BQ834710	1	*Pso-cathb*-1	Cathepsin B-like cysteine protease	*Bombyx mori*	$1{\cdot}5\ e^{-49}$	204	48	63
BQ834913	1	*Pso-cathb*-2	Cathepsin B-like cysteine protease	*C. elegans*	$6{\cdot}1\ e^{-16}$	57	72	84
BQ834906	1	*Pso-cathl*-1	Cathepsin L-like cysteine protease	*Sitophilus zeamais*	$1{\cdot}3\ e^{-82}$	239	65	76
Free-radical scavenging enzymes								
BQ834608	4	*Pso-gst*-2	Glutathione S-transferase	*D. melanogaster*	$9{\cdot}8\ e^{-21}$	141	45	65
BQ834873	1	*Pso-tpx*-1	Thioredoxin peroxidase	*Globodera rostochiensis*	$9{\cdot}4\ e^{-73}$	234	75	87
BQ834914	1	*Pso-tpx*-2	Thioredoxin peroxidase	*Homo sapiens*	$8{\cdot}8\ e^{-68}$	210	68	85
BQ834951	1	*Pso-tpx*-3	Thioredoxin peroxidase	*D. melanogaster*	$5{\cdot}9\ e^{-62}$	196	85	87
BQ834990	1	*Pso-sod*-1	Superoxide dismutase	*Oryza sativa*	$1{\cdot}2\ e^{-49}$	149	70	79
Others of interest								
BQ834600	19	*Pso-lim*-1	LIM domain containing protein	*C. elegans*	$5{\cdot}5\ e^{-27}$	106	59	73
BQ834722	2	*Pso-cpi*-1	Cysteine protease inhibitor	*Bos taurus*	$6{\cdot}3\ e^{-10}$	155	42	58
BQ834901	2	*Pso-pkc*-1	Protein kinase C	*Aplysia californica*	$2{\cdot}3\ e^{-37}$	173	48	65
BQ835048	2	*Pso-cytoc*-1	Cytochrome C oxidase	*Homo sapiens*	$1{\cdot}3\ e^{-16}$	218	26	51
BQ834616	1	*Pso-pdi*-1	Protein disulphide isomerase	*Mus musculus*	$2{\cdot}2\ e^{-30}$	213	62	76
BQ834761	1	*Pso-fsp*-1	Fungal stress protein	*Rhizopus nigricans*	$4{\cdot}0\ e^{-40}$	238	43	64
BQ834767	1	*Pso-adh*-1	adenosylhomocyteinase	*C. elegans*	$2{\cdot}8\ e^{-71}$	188	77	85
BQ834801	1	*Pso-prot*-1	Proteasome subunit beta type 4 precursor	*Xenopus laevis*	$1{\cdot}8\ e^{-46}$	238	54	73
BQ834896	1	*Pso-ier*-1	Lymphocyte IgE receptor	*Homo sapiens*	$3{\cdot}8\ e^{-10}$	251	33	47
BQ834927	1	*Pso-cut*-1	Rigid cuticular protein	*Araneus diadematus*	$2{\cdot}8\ e^{-09}$	165	50	55
BQ834991	1	*Pso-alg2*-1	Programmed cell death protein	*Mus musculus*	$2{\cdot}3\ e^{-53}$	184	57	77
BQ835077	1	*Pso-aladh*-1	Haem biosynthesis enzyme	Fission yeast	$8{\cdot}1\ e^{-37}$	100	38	54

(*Ribosome-associated proteins* N = 36).

and extracellular digestion, in the case of the latter to aid the penetration of collagen and keratin barriers in the skin (Stewart, 1995).

The present study was initiated with the aim of rapidly identifying further potential allergens and other proteins of importance to lesion formation during *P. ovis* infestation using expressed sequence tag (EST) analysis. In this approach, sequences derived from randomly selected cDNAs can be used to define the genes expressed by an organism. This method has been used with success in the identification of genes from a variety of parasites including the cattle tick, *Boophilus microplus* (Crampton *et al*. 1998), the ovine gastrointestinal nematode, *Haemonchus contortus* (Hoekstra *et al*. 2000), the filarial nematodes, *Brugia malayi* (Blaxter *et al*. 2002) and *Onchocerca volvulus* (Lizotte-Waniewski *et al*. 2000) and the human hookworm, *Necator americanus* (Daub *et al*. 2000).

MATERIALS AND METHODS

cDNA library preparation and expressed sequence tag generation

P. ovis mites (700 mg, mixed sex) were harvested from the skin of experimentally infected sheep (Smith *et al*. 2002) and contaminating skin debris removed by extensive washing. Messenger RNA was prepared from *P. ovis* mites using an mRNA extraction kit (Stratagene, UK) and a cDNA library was constructed in Lambda TriplEx2 cloning vector according to the manufacturer's instructions (Clontech). The library was plated on *XL-1*-Blue cells and recombinant clones picked at random. The library titre was $1{\cdot}6 \times 10^6$ pfu/ml with 89% recombinant phage as judged by blue/white colour selection. The cDNA inserts of randomly selected clones were amplified by PCR using the universal M13 forward and reverse vector primers. Insert cDNAs >500 bp (75% of the total) were selected for sequencing. The PCR products were cleaned and sequence analyses conducted using ABI rhodamine dye terminators and an ABI 377 automated sequencer (Daub *et al*. 2000).

Bioinformatics

Sequences were checked, vector and poor quality 3′ sequences removed using automated methods applied to the *Brugia* EST project and then compared to the public databases (GenBank non-redundant nucleotide and protein databases and dbEST) using the BLAST algorithms (Altschul *et al*. 1990). Sequences were clustered on the basis of homology using CLOBB v1.0 (Parkinson, Guiliano & Blaxter, 2002). Briefly, CLOBB is an iterative clustering method where sequences are clustered on the basis of BLAST similarity. It tracks cluster-specific events such as merging, identifies 'superclusters' of related clusters and avoids expansion of chimeric clusters (Parkinson *et al*. 2002). Putative function was assigned where possible, all conducted within the quality control parameters outlined by Daub *et al*. (2000). The cluster analysis is available at http://nema.cap.ed.ac.uk/Chelicerates/ChelDB.php. Motif analysis was not conducted except in specific instances, outlined below, where signal peptides or potential glycosylation sites were sought. Multiple sequence alignments were conducted using Clustal X (Thompson *et al*. 1997), phylogenetic trees constructed using Phylip 3.5 with the default parameters and visualized with TreeView (Page, 1996).

RESULTS AND DISCUSSION

This study was initiated with the aim of rapidly identifying further potential allergens and other proteins of potential importance to lesion formation during *P. ovis* infestation. Only a group 2 allergen homologue had been cloned and sequenced from *P. ovis* (Temeyer *et al*. 2002) prior to this study. Despite the limited number of ESTs analysed here, the present study identified 45 ESTs (9% of the total) encoding proteins with similarity to known allergens previously identified in house dust, storage and forage mite species (Table 1). These mite species contain several allergens with interspecies similarities which lead to immunological cross-reactivity. The allergens can be loosely divided into 2 categories, namely those with or without enzyme activity (Stewart, 1995). Many of the former are proteases which are derived from cells lining the gastrointestinal tract of the mite and are present in faecal extracts (Stewart, 1995). Proteases may initiate the lesion and contribute to nutrient provision for the mite. The non-enzymatic allergens include heat shock proteins, fatty acid binding proteins and structural proteins such as tropomyosin (reviewed by Stewart, 1995; Pomes, 2002). A number of ESTs encoded free-radical scavenging enzymes which may help the mite evade or modulate the host immune response. These data emphasize the value of EST analysis, even on a small scale, to identify parasite genes encoding proteins of potential importance in the host–parasite interaction.

In total 507 sequences were clustered, including 23 previously generated *P. ovis* sequences downloaded from Genbank. These formed 280 clusters of which 49 had >1 sequence with 24 showing significant Blast X homology to another protein in the databases. There were 231 sequences which appeared on one occasion and 109 of these showed significant Blast X homology to other sequences in the databases. The unequivocal definition of highly expressed genes by cluster analysis is not possible because of the small size of the present dataset. However, Daub *et al*. 2000 noted that early patterns of abundance in ESTs from the nematode *Brugia*

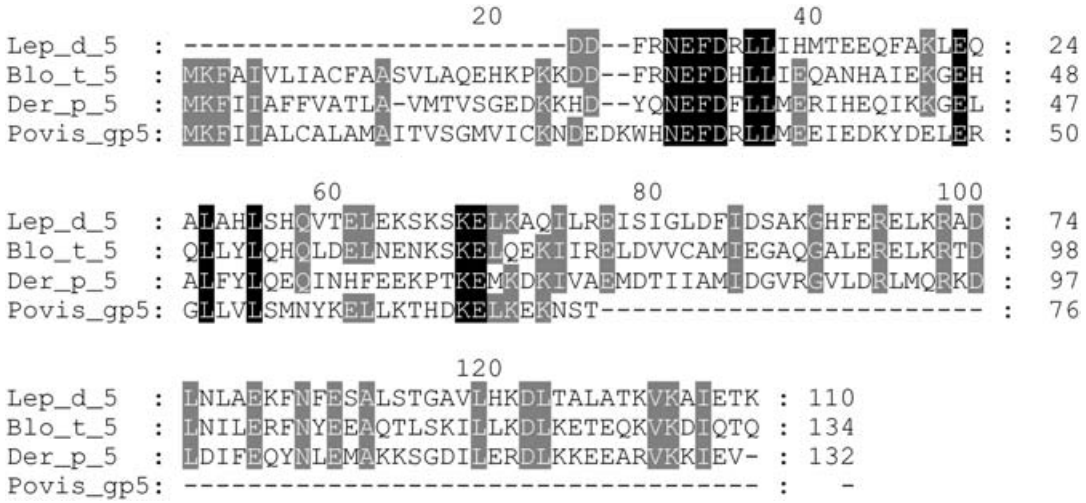

Fig. 1. Alignment of the group 5 allergens. Residues shaded in black are identical in all 4 sequences, those shaded in grey are present in 3 of the 4. –, Gaps inserted to improve the alignment. The sequences used and the GenBank accession numbers are: *Lep d* 5, *Lepidoglyphus destructor*, Q9U5P2; *Blo t* 5, *Blomia tropicalis*, Q96870; *Der p* 5, *Dermatophagoides pteronyssinus*, P14004; Povis_gp5, Pso_Der p V identified from EST analysis, BQ834975.

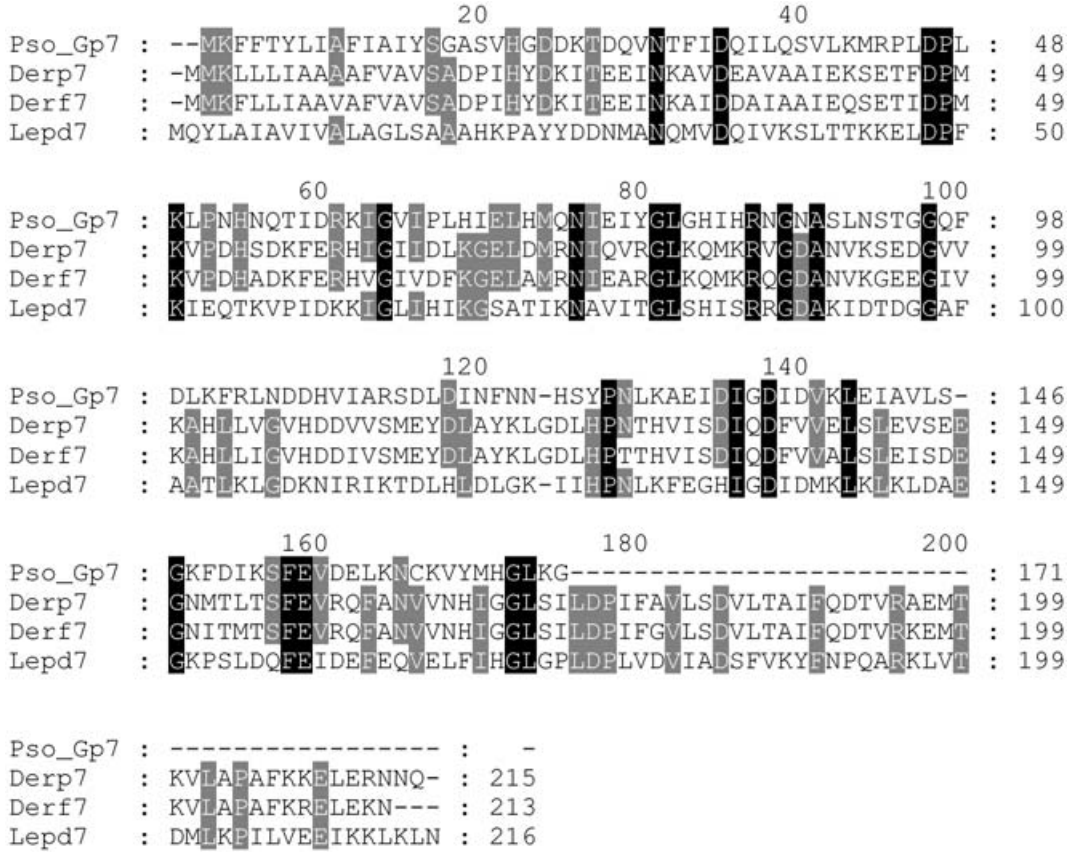

Fig. 2. Alignment of the group 7 allergens. Residues shaded in black are in all 4 sequences, those shaded in grey in 3 of the 4. –, Gaps inserted to improve alignment. Sequences used and their GenBank accession numbers were: Pso_Gp7, *P. ovis* group 7 allergen homologue identified from the EST analysis, BQ834778; Lep d 7; *Lepidoglyphus destructor*, Q9U1G2; Der p 7; *Dermatophagoides pteronyssinus*, P49273; Der f 7; *D. farinae*, Q26456.

malayi were, in general terms, confirmed by more extensive sequencing. Nine ESTs encoded homologues of the house dust mite group 1 allergens, which are cysteine proteases (Chua *et al*. 1988). In *Dermatophagoides pteronyssinus*, Der p 1, the homologue of Der F A in *Dermatophagoides farinae*, is found in high concentrations in mite faeces and is present in the mite gut epithelium (Tovey & Baldo, 1990). Whilst probably functioning as a digestive enzyme, there is now a large body of evidence supporting

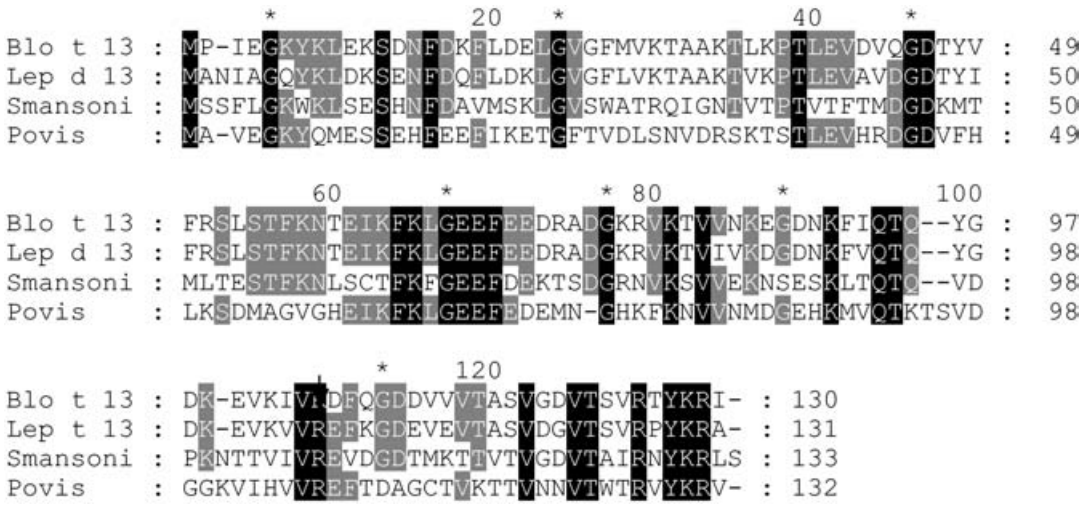

Fig. 3. Alignment of the group 13 (fatty acid binding) allergens. Residues shaded in black are identical, residues shaded in grey are in 3 of the 4. –, Gaps inserted to improve alignment. The sequences used and their GenBank accession numbers are as follows: Blo t 13, *Blomia tropicalis*, Q17284; Lep d 13, *Lepidoglyphus destructor*, Q9U5P1; S. mansoni, *Schistosoma mansoni* fatty acid binding protein, P29498; Povis, *P. ovis* fatty acid binding protein homologue identified from EST analysis, BQ834621. * Glycines involved in β-sheet alignment. ↓ Conserved arginine which participates in electrostatic interaction with the carboxyl group of the fatty acid.

the view that this proteolytic activity contributes to allergenicity (reviewed by Pomes, 2002).

The present study also identified 3 distinct cysteine proteases encoding ESTs (Pso CathB1, Pso CathB2 and Pso CathL1) each represented once and not previously identified in other mite species (Table 1). In helminths, proteases of these types have been implicated in, amongst other functions, digestion and in the penetration of host tissue barriers (Tort *et al*. 1999), some being found in the *in vitro* excretions/secretions indicating a role in extracorporeal digestion. Hence, these proteases may also contribute to the disease syndrome and are worthy of further analysis. An earlier study used various biochemical analyses to demonstrate the presence of aspartyl and metalloproteases in mite extracts and the authors suggested that these enzymes may be important in mite physiology or the aetiology of the disease (Kenyon & Knox, 2002). No ESTs encoding these classes of protease were identified here, a fact which may simply reflect the small number of sequences sampled.

The group 2 allergen sequence recently identified from *P. ovis* (Temeyer *et al*. 2002) was the most abundant in the dataset, with 20 ESTs in the cluster. Sequence analysis of the group 2 allergens from house dust mites suggests they are non-glycosylated proteins which contain leader peptide sequences that are cleaved to yield the mature protein. The mature proteins are approximately 14 kDa and contain 3 disulphide bonds (Nishiyama *et al*. 1993) which are also present in the *P. ovis* homologue (Temeyer *et al*. 2002). These allergens are highly abundant in whole body extracts of the dust mites and are recognized by 80–90% of mite-allergic individuals (Heymann *et al*. 1989), but have an as yet undefined function in mite physiology. However, Der p 2 has 35% sequence similarity with a human epididymal gene product suggesting a possible role in male reproduction (Mueller, Benjamin & Rule, 1998).

Another cluster of 2 ESTs showed 40% identity with a group V allergen from *Lepidoglyphus destructor*. The Group V allergens are recognized by about 50% of mite-allergic individuals (Tovey *et al*. 1989). These allergens have been identified from *D. pteronyssinus* and *B. tropicalis* but a homologue in *D. farinae* mites has not yet been identified. These allergens range in molecular weight from 14 to 17 kDa and their function is, as yet, unknown. Signal peptide analysis indicates a possible signal peptide cleavage site between positions 18 and 19: VSG-MV. The alignment (Fig. 1) indicates 1 region of close homology between all 4 sequences (residues 31–37, *P. ovis* numbering). Searches using a variety of protein domain servers available on the web failed to ascribe any functional significance to this region.

A cluster comprising 3 ESTs showed closest homology to an allergen, *Lep d* 7, secreted from the storage mite *L. destructor* (Eriksson *et al*. 2001). This allergen is a close homologue of the group 7 allergens from *Dermatophagoides* spp. (Fig. 2). Their biological function is undefined; however, they are known to range from 26 to 31 kDa in size and bind IgE in 50% of sera from allergic people (Shen *et al*. 1995). The coding sequences for these proteins contain leader peptides indicative of secretion as well as a predicted *N*-linked glycosylation site (Shen *et al*. 1993). The *P. ovis* sequence contains 4 potential *N*-linked glycosylation sites, none of which are conserved with other Type 7 allergens, as well as

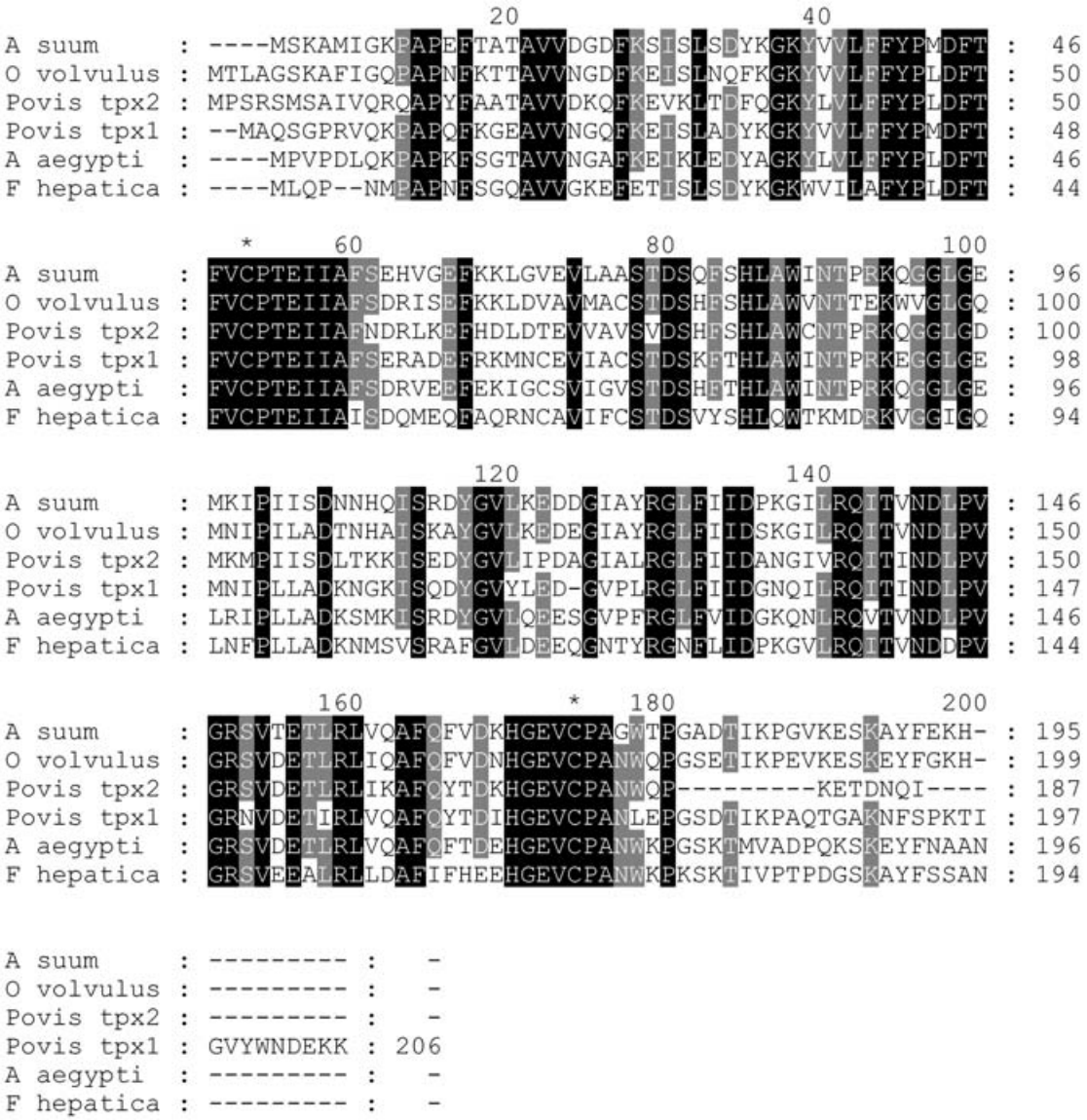

Fig. 4. Alignment of the thioredoxin peroxidase protein sequences. Residues shaded in black are present in all the sequences, those shaded in grey in 5 of the 6. –, Gaps inserted to improve alignment. * Conserved cysteines which contribute to homodimer formation. Sequences used and their GenBank accession numbers are: *Ascaris suum*, Q9NL98; *Onchocerca volvulus*, O44366; *Aedes aegypti*, Q8WSF6; *Fasciola hepatica*, P91883. *Psoroptes ovis* tpx 1 and 2 were identified from the EST analysis, BQ834873 and BQ834914, respectively.

a predicted signal peptide cleavage site at amino acid 22.

Four ESTs encoded a homologue of a mite group 13 allergen, *Blo t* 13, a fatty acid-binding protein (FABP), isolated from the mite *B. tropicalis* (Fig. 3, Caraballo *et al*. 1997). The group 13 allergens have also been identified from the dust mites *Acarus siro* (Eriksson *et al*. 1999) and *L. destructor* (Eriksson *et al*. 2001), and bind IgE in only 11% of sera from mite allergic individuals (Caraballo *et al*. 1997). FABPs are involved in general lipid metabolism acting as intracellular transporters of hydrophobic metabolic intermediates and as carriers of lipids between membranes. Fatty acid binding proteins have been identified in the midgut of larval *Manduca sexta*, the tobacco hornworm (Smith *et al*. 1992), possibly indicative of a function in lipid uptake from the gut. The *P. ovis* sequence does not contain a putative signal peptide cleavage site indicating it is not secreted but contains a potential *N*-linked glycosylation site at amino acids 122–125. Six of seven glycine residues involved in the β-sheet orientation of fatty acid binding proteins (Sacchettini, Banaszak & Gordon, 1992) are conserved in the *P. ovis* sequence (* Fig. 3) and an arginine (arrowed in Fig. 3), which participates in electrostatic interaction with the carboxyl group of the fatty acid (Sacchettini *et al*. 1992), is conserved in all the sequences in the alignment.

Three other putative allergens, namely tropomyosin, paramyosin and a heat shock protein, were present as single ESTs (Table 1). Tropomyosin is

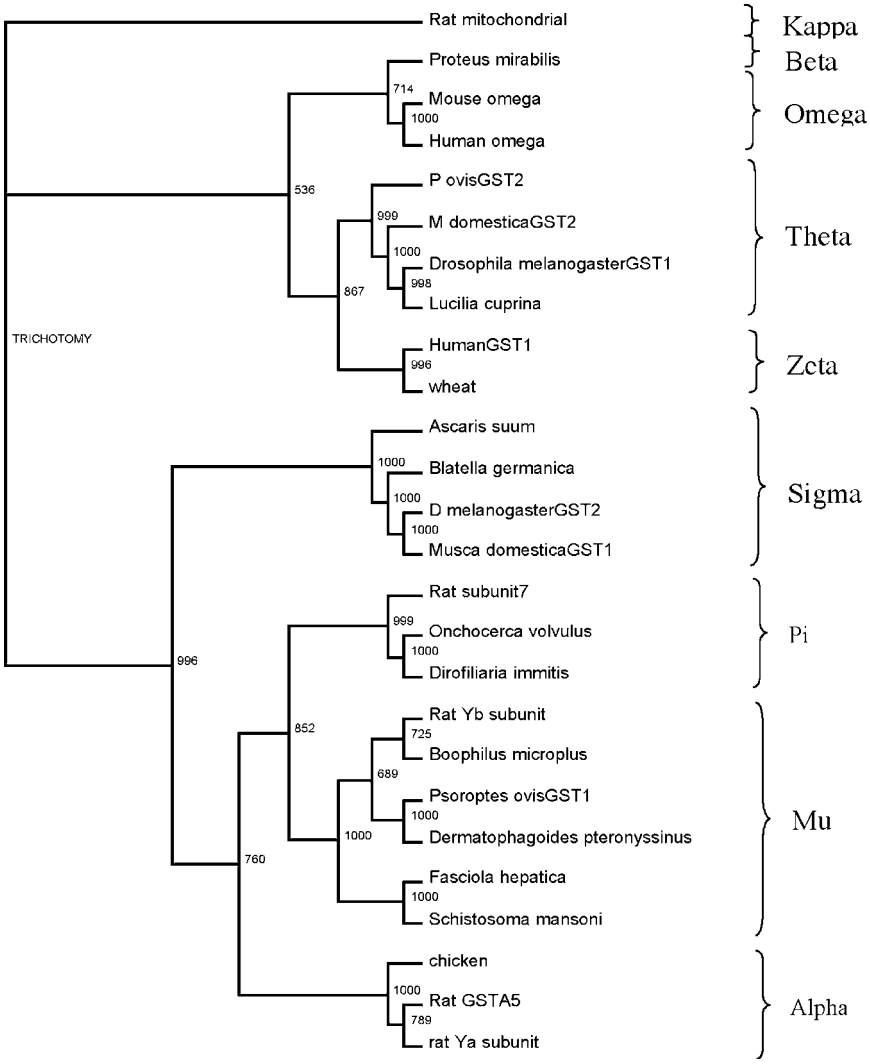

Fig. 5. Phylogenetic analysis of GST protein sequences. Annotation to the right of the diagram denotes the class of GST. The bootstrap values are indicated at the nodes of the tree. The GenBank accession numbers are as follows: Rat mitochondrial, P24473; *Proteus mirabilis*, P15214; Mouse omega, U80819; Human omega, AF212303; *P. ovis* GST2, GST homologue identified in the EST analysis, BQ834608; *Musca domestica* GST2, P46431; *Drosophila melanogaster* GST1, P20432; *Lucilia cuprina*, P42860; Human GST1, AAB96392; Wheat AAD09190; *Ascaris suum*, CAA53218; *Blatella germanica*, O18598; *D. melanogaster* GST2, P41043; *M. domestica* GST1, AAA03434; Rat subunit 7, P04906; *Onchocerca volvulus*, AAA53575; *Dirofiliaria immitis*, P46426; Rat Yb subunit, P04905; *Boophilus microplus*, AAD15991; *P. ovis* GST1, AAF19264; *Dermatophagoides pteronyssinus*, P46419; *Fasciola hepatica*, P56598; *Schistosoma mansoni*, P35661; chicken, Q08393; Rat GSTA5, A54858; Rat Ya subunit, P00502.

found in most metazoans and is a structural protein that is involved in the regulation of calcium flux in muscles. Tropomyosin (Der f 10) has been isolated from *D. farinae* (Aki *et al*. 1995) and binds IgE in about 80% of mite allergenic individuals. Paramyosin has been assigned as a Group 11 allergen and has a relatively high molecular weight (~98 kDa). It is recognized by greater than 80% of sera from mite allergic individuals (Tsai *et al*. 1998). Paramyosin is one of the primary candidate proteins for vaccine development against schistosomiasis (McManus *et al*. 2001) and tropomyosin induces partial protective immunity in sheep against gastrointestinal nematodes (Cobon *et al*. 1989).

The heat shock protein identified in this study was homologous to the small heat shock protein family from *Caenorhabditis elegans*. Heat shock proteins (hsp) are a highly conserved family of proteins that are present in both pro- and eukaryotes and are thought to have play a role in protein folding.

Three types of free-radical scavenging enzyme were identified in this study, namely superoxide dismutase (SOD), thioredoxin peroxidase (TPX) and glutathione S-transferase (GST). Free-radical scavenging enzymes neutralize potentially toxic free-radicals such as the superoxide anion, hydrogen peroxide and the hydroxyl radical (Henkle-Dührsen & Kampkötter, 2001). The presence of the free-radical scavenging enzymes in parasitic organisms has been linked with immunomodulation.

The SOD identified here was homologous to the copper/zinc form of the enzyme. It lacked a

predicted signal peptide indicating it is a cytosolic and is unlikely to be involved in the pathogenesis of the disease.

The thioredoxin peroxidases or peroxidoxins are a family of antioxidant enzymes which have only recently been identified and are characterized by having no cofactors (i.e. metals) (McGonigle, Curley & Dalton, 1997). These enzymes primarily neutralize hydrogen peroxidase thus preventing a build-up of the toxic hydroxyl radical. TPX has not been identified previously in arthropod parasites. Here 3 TPX ESTs were identified, 2 being essentially identical (Pso-Tpx1 and Pso-Tpx3), the third (Pso-Tpx2) being quite distinct (Fig. 4). None of these sequences contained a predicted signal peptide cleavage site indicating they are not secreted. There are 2 main forms of TPX (Henkle-Duhrsen & Kampkotter, 2001), the 1-Cys and the 2-Cys types and those identified were the 2-cys type. These enzymes are characterized by the presence of a conserved FVCP sequence around the first cysteine, and a second conserved residue in the C-terminal region, separated from the first by approximately 120 residues (Henkle-Dührsen & Kampkötter, 2001). These conserved cysteines contribute to homodimer formation and function. The presence of more than 1 distinct TPX in the EST dataset suggests that these enzymes may be part of a multi-gene family and may be important in mite physiology.

Glutathione S-transferases (GST) are ubiquitous detoxifying enzymes which catalyse the conjugation of reduced glutathione with a number of electrophiles. In helminths, GSTs usually occur as several isoforms and play a central role in the parasite detoxification system including host-derived antiparasite factors (Brophy & Pritchard, 1994). There are 9 classes of cytosolic GST defined by a number of factors including substrate specificity, sequence-similarity, and phylogenetic analyses. A GST homologue from *D. pteronyssinus* has been shown to be allergenic (O'Neill *et al*. 1995) and forms group 8 mite allergens. These allergens are approximately 26 kDa in size, and the recombinant molecule is recognized by approximately 40% of mite allergenic individuals (O'Neill, Donovan & Baldo, 1995). A mu-class GST homologue was recently identified in *P. ovis* (*P. ovis* GST1) (Lee *et al*. 2002). A theta-class GST, *P. ovis* GST2, was identified in the ESTs (Fig. 5).

A large proportion (47·5%) of the ESTs were not significantly similar to any protein in the public databases and were classed as novel sequences. Of the 23 clusters that encoded novel proteins, 6 comprised 8 or more ESTs suggesting relatively abundant mRNAs and thus protein products. Of these six clusters, 4 had predicted signal peptides suggesting that they could be secreted by the mites and therefore be important in the pathogenesis of the disease. Other proteins of potential interest identified included a cystatin (a cysteine protease inhibitor), a LIM domain containing protein which may be involved in myogenesis and a homologue of a lymphocyte IgE receptor, the latter being involved in IgE production and the differentiation of B-cells.

In summary, this study has identified several ESTs encoding putative proteins, including allergens, proteases and free-radical scavenging enzymes, which are likely to contribute to lesion formation and disease pathogenesis. These gene products are worthy of further investigation including detailed immunological analyses and possible vaccination studies given that lesion size and mite numbers can be reduced by vaccination of sheep with mite extracts prior to challenge infection (Smith *et al*. 2002).

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