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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 (Prueitt, 1999; Temeyer, Soileau & Prueitt, 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 aminopeptidases (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.)

<table>
<thead>
<tr>
<th>Description and Accession no.</th>
<th>No. of ESTs</th>
<th>Gene name</th>
<th>Putative function</th>
<th>Homologue in</th>
<th>Blast X E-value</th>
<th>ORF length (aa)</th>
<th>Homology (%)</th>
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<tr>
<td><strong>Allergens</strong></td>
<td></td>
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<tr>
<td>BQ834604</td>
<td>20</td>
<td><em>Pso-o-II</em></td>
<td>Group II allergen</td>
<td><em>P. ovis</em></td>
<td>1.2 e^{-72}</td>
<td>160</td>
<td>99 99</td>
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<td>BQ834615</td>
<td>9</td>
<td><em>Pso-der F A-1</em></td>
<td>Secreted cysteine protease</td>
<td><em>Dermatophagoides farinae</em></td>
<td>2.1 e^{-73}</td>
<td>231</td>
<td>69 79</td>
</tr>
<tr>
<td>BQ834608</td>
<td>4</td>
<td><em>Pso-gst-2</em></td>
<td>Glutathione S-transferase</td>
<td><em>Drosophila melanogaster</em></td>
<td>9.8 e^{-21}</td>
<td>141</td>
<td>45 65</td>
</tr>
<tr>
<td>BQ834621</td>
<td>4</td>
<td><em>Pso-fabp-1</em></td>
<td>Fatty acid binding protein</td>
<td><em>Blomia tropicalis</em></td>
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<td>134</td>
<td>35 53</td>
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<td>BQ834778</td>
<td>3</td>
<td><em>Pso-gp7-1</em></td>
<td>Unknown, group 7 allergen</td>
<td><em>Lepidoglyphus destructor</em></td>
<td>1.2 e^{-22}</td>
<td>178</td>
<td>35 57</td>
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<td>BQ834973</td>
<td>2</td>
<td><em>Pso-der p V-1</em></td>
<td>Der p V IgE binding allergen</td>
<td><em>D. pteronyssinus</em></td>
<td>8.0 e^{-35}</td>
<td>134</td>
<td>40 64</td>
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<td>BQ834874</td>
<td>1</td>
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<td>Tropomyosin</td>
<td><em>D. farinae</em></td>
<td>7.6 e^{-94}</td>
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<td><em>Pso-paramy-1</em></td>
<td>Paramyosin</td>
<td><em>Sarcoptes scabiei</em></td>
<td>1.5 e^{-58}</td>
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<td>1</td>
<td><em>Pso-hsp-1</em></td>
<td>Heat shock protein</td>
<td><em>Caenorhabditis elegans</em></td>
<td>9.2 e^{-13}</td>
<td>149</td>
<td>41 58</td>
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<tr>
<td><strong>Cysteine proteases</strong></td>
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<tr>
<td>BQ834615</td>
<td>9</td>
<td><em>Pso-der F A-1</em></td>
<td>Secreted cysteine protease</td>
<td><em>D. farinae</em></td>
<td>2.1 e^{-73}</td>
<td>231</td>
<td>69 79</td>
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<td>BQ834710</td>
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<td><em>Pso-cathb-1</em></td>
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<td><em>Bombyx mori</em></td>
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<td>Cathepsin B-like cysteine protease</td>
<td><em>C. elegans</em></td>
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<td>72 84</td>
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<td><em>Pso-cathl-1</em></td>
<td>Cathepsin L-like cysteine protease</td>
<td><em>Sitophilus zeamais</em></td>
<td>1.3 e^{-82}</td>
<td>239</td>
<td>65 76</td>
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<td><strong>Free-radical scavenging enzymes</strong></td>
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<td>BQ834608</td>
<td>4</td>
<td><em>Pso-gst-2</em></td>
<td>Glutathione S-transferase</td>
<td><em>D. melanogaster</em></td>
<td>9.8 e^{-31}</td>
<td>141</td>
<td>45 65</td>
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<td>BQ834873</td>
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<td><em>Pso-tpx-1</em></td>
<td>Thioredoxin peroxidase</td>
<td><em>Globodera rostochiensis</em></td>
<td>9.4 e^{-23}</td>
<td>234</td>
<td>75 87</td>
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<td><em>Pso-tpx-2</em></td>
<td>Thioredoxin peroxidase</td>
<td><em>Homo sapiens</em></td>
<td>8.8 e^{-48}</td>
<td>210</td>
<td>68 85</td>
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<tr>
<td>BQ834951</td>
<td>1</td>
<td><em>Pso-tpx-3</em></td>
<td>Thioredoxin peroxidase</td>
<td><em>D. melanogaster</em></td>
<td>5.9 e^{-62}</td>
<td>196</td>
<td>85 87</td>
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<tr>
<td>BQ834990</td>
<td>1</td>
<td><em>Pso-sod-1</em></td>
<td>Superoxide dismutase</td>
<td><em>Oryza sativa</em></td>
<td>1.2 e^{-49}</td>
<td>149</td>
<td>70 79</td>
</tr>
<tr>
<td><strong>Others of interest</strong></td>
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<td>BQ834600</td>
<td>19</td>
<td><em>Pso-lim-1</em></td>
<td>LIM domain containing protein</td>
<td><em>C. elegans</em></td>
<td>5.5 e^{-27}</td>
<td>106</td>
<td>59 73</td>
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<td>BQ834722</td>
<td>2</td>
<td><em>Pso-cpi-1</em></td>
<td>Cysteine protease inhibitor</td>
<td><em>Bos taurus</em></td>
<td>6.3 e^{-10}</td>
<td>155</td>
<td>42 58</td>
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<tr>
<td>BQ834901</td>
<td>2</td>
<td><em>Pso-phi-1</em></td>
<td>Protein kinase C</td>
<td><em>Aplysia californica</em></td>
<td>2.3 e^{-37}</td>
<td>173</td>
<td>48 65</td>
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<tr>
<td>BQ835048</td>
<td>2</td>
<td><em>Pso-cytoc-1</em></td>
<td>Cytochrome C oxidase</td>
<td><em>Homo sapiens</em></td>
<td>1.3 e^{-18}</td>
<td>218</td>
<td>26 51</td>
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<td>BQ834616</td>
<td>1</td>
<td><em>Pso-pdi-1</em></td>
<td>Protein disulphide isomerase</td>
<td><em>Mus musculus</em></td>
<td>2.2 e^{-38}</td>
<td>213</td>
<td>62 76</td>
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<tr>
<td>BQ834761</td>
<td>1</td>
<td><em>Pso-fsp-1</em></td>
<td>Fungal stress protein</td>
<td><em>Rhizopus nigricans</em></td>
<td>4.0 e^{-40}</td>
<td>238</td>
<td>43 64</td>
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<tr>
<td>BQ834767</td>
<td>1</td>
<td><em>Pso-adh-1</em></td>
<td>adenosylhomocysterinase</td>
<td><em>C. elegans</em></td>
<td>2.8 e^{-71}</td>
<td>188</td>
<td>72 85</td>
</tr>
<tr>
<td>BQ834801</td>
<td>1</td>
<td><em>Pso-pro-1</em></td>
<td>Proteasome subunit beta type 4 precusor</td>
<td><em>Xenopus laevis</em></td>
<td>1.8 e^{-46}</td>
<td>238</td>
<td>54 73</td>
</tr>
<tr>
<td>BQ834896</td>
<td>1</td>
<td><em>Pso-ier-1</em></td>
<td>Lymphocyte IgE receptor</td>
<td><em>Homo sapiens</em></td>
<td>3.8 e^{-10}</td>
<td>251</td>
<td>33 47</td>
</tr>
<tr>
<td>BQ834927</td>
<td>1</td>
<td><em>Pso-cut-1</em></td>
<td>Rigid cuticular protein</td>
<td><em>Araneus diadematus</em></td>
<td>2.8 e^{-39}</td>
<td>165</td>
<td>50 55</td>
</tr>
<tr>
<td>BQ834991</td>
<td>1</td>
<td><em>Pso-alg2-1</em></td>
<td>Programmed cell death protein</td>
<td><em>Mus musculus</em></td>
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<td>184</td>
<td>57 77</td>
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<tr>
<td>BQ835077</td>
<td>1</td>
<td><em>Pso-aladh-1</em></td>
<td>Haem biosynthesis enzyme</td>
<td><em>Fission yeast</em></td>
<td>8.1 e^{-47}</td>
<td>100</td>
<td>38 54</td>
</tr>
</tbody>
</table>

(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-Waniekiewski 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 1·6 × 10⁶ 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*...
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
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
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...
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. Para-myosin 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). Para-myosin 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 & Kampköttner, 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 peroxide 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-Duhrs & Kampkötter, 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-Duhren & 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 (O’Neill et al. 1995). A theta-class GST, was identified in the ESTs (Fig. 5). These genes are characterized by the presence of a conserved GST1 (1998). Expressed sequenced tags and new genes from 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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REFERENCES


Gene expression in Psoroptes ovis


