

Identification of tropomyosin, paramyosin and apolipophorin/vitellogenin as three major allergens of the sheep scab mite, *Psoroptes ovis*

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SUMMARY

Analysis by one-dimensional (1-D) SDS-PAGE/Western blotting of whole mite extract (larval and adult stages) with sheep sera taken 0–84 days after infection with the sheep scab mite, Psoroptes ovis revealed a progressive IgE antibody response, with a dominant high molecular weight allergen (MW 180 kDa) detected early during infection. Further analysis by two-dimensional (2-D) SDS-PAGE/Western blotting with post-infection sera, revealed a more complex picture with numerous (> 20) IgE reactive spots. Trypsin digest and Maldi-ToF analyses of these spots identified two house dust mite allergen homologues, namely tropomyosin (Der p 10) and paramyosin (Der p 11), and analysis of a third spot indicated an apolipoprotein-like IgE reactive protein (Der p 14). Further 1-D and 2-D SDS/Western blotting, with specific antibodies to the house dust mite allergens Der p 10, 11, and to the IgE reactive peptide of the high molecular weight house dust mite allergen, Der p 14 (vitellogenin/apolipophorin), provided firm evidence for the presence of these three allergens in extracts of the Psoroptes mite. These studies show for the first time that homologues of the house dust mite 10, 11 and 14 group allergens are represented as immunodominant allergens of the sheep scab mite, and may represent important vaccine candidates.

Keywords allergen, Der p 10, Der p 11, Der p 14, *Psoroptes ovis*, sheep scab

INTRODUCTION

Sheep scab, caused by the mite *Psoroptes ovis*, is perhaps, the most important ectoparasitic disease of sheep. In the UK the prevalence of this disease has escalated to become endemic in hill and lowland sheep. The mites live entirely on the skin surface and, unlike *Sarcoptes scabiei*, do not burrow into the epidermis but induce a profound exudative skin reaction. The disease is characterized by extensive dermatitis, yellow scab formation and intense itching (1). Studies on the immune response to infection have shown that previous infection moderates the course of lesion development following a challenge infection, indicating the presence of a protective immune reaction to the mites (2). Histopathological analysis of the dermal lesions has demonstrated a pronounced local inflammatory reaction, involving mast cells, eosinophils and T helper cells, indicating a vigorous Th2 response to mite antigens (1,2). Infection also generates systemic IgG and IgE antibodies (3), and skin tests of mite antigen readily induce a positive wheal skin reaction that is associated with a dermal inflammatory cell influx (4). This reaction is significantly reduced in passive cutaneous assays where the sera have been heated at 56°C for 1 h, suggesting that IgE antibody reactions are important in the immunopathology of the scab lesion. Analysis of these IgE responses by Enzyme Linked Immunosorbent Assays (ELISA) have shown that blood levels of IgE antibodies increase through the duration of a primary infection, and an anamnestic IgE response occurs following secondary challenge (3).

These previous studies have therefore implicated *P. ovis* allergens in the development of the pathology and/or the development of immunity to infection. Previous analysis of Expressed Sequence Tag (EST) has shown the presence of

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transcripts encoding several allergens with close homology to house dust mite allergens (5). In addition, a Der p 1 homologue from *P. ovis*, termed Pso o 1, which encodes a cysteine proteinase, has been fully sequenced and expressed as recombinant protein (6), and sera from sheep infested with *P. ovis* contained IgE that recognized the recombinant protein. In the current study, we have continued our analysis of the immune response to sheep scab and have identified three house dust mite allergen homologues that appear to be immunodominant allergens in sheep scab infection.

MATERIALS AND METHODS

Animals

Nine *P. ovis*-naïve × Suffolk sheep aged 1–2 years were infected with 25–50 ovigerous *P. ovis* mites as described previously (2). Blood samples were collected into vacutainer tubes, containing no anticoagulant (Becton Dickinson, Franklin Lakes, NJ, USA), by jugular venipuncture, and the serum was harvested. Sera were taken before infection and at 2 weeks and 12 weeks post-infection, when the sheep were treated with an endectocide (Ivomec, MSB Agvet, 200 µg/kg). Eleven weeks after this treatment, animals were challenged with the same mite dose. Sera were collected 1 day prior to this challenge, and at 2 and 6 weeks post-challenge.

Antigens

Psoroptes ovis whole-mite extracts (WME) were prepared as previously described (2). Briefly, mites (adults, nymphs and larvae) were collected from donor infestations in sheep, and the live mites washed by vortexing for 5 min in ice-cold phosphate buffered saline (PBS, pH 7.2), followed by 1% sodium dodecyl sulphate (SDS) at room temperature and then 10 washes in ice-cold PBS. Mites were then homogenized and sonicated in ice-cold PBS, and this homogenate was centrifuged at 10 000 *g* for 10 min (at 4°C) and the supernatant [whole mite extract (WME)] harvested, aliquoted and stored at –70°C. Protein content of the supernatant was determined, in accordance with the manufacturer's instructions, with BCA Protein Assay Kit (Pierce, IL, USA).

Anti-allergen antibodies

Mouse antibodies to purified tropomyosin from the tropical house dust mite, *Blomia tropicalis* (Blo t 10; group 10 mite antigen) were prepared as described previously (7). Mouse antibodies to paramyosin from *B. tropicalis* (Blo t 11; group 11 mite allergen) were generated by DNA vaccination, as described previously (8). Mouse polyclonal sera and two monoclonal antibodies (clones 3F1 and 7A9), to a recom-

binant peptide of Der p 14 (1–224), which represents a conserved fragment with a high frequency of IgE binding (9), were employed to detect a Der p 14 homologue in *P. ovis* WME. Partially purified peptide Der p 14 (1–224) was also employed in specificity studies.

1-D SDS-PAGE and immunoblotting

Initial studies employed 12% tris-glycine/SDS gels and running buffer (Novex, San Diego, CA, USA), but later and improved SDS-PAGE separation of *P. ovis* WME was performed under non-reducing conditions utilizing gradient 4%–12% NuPAGE Novex Bis-Tris gels (Novex, Invitrogen) employing NuPAGE MES SDS running buffer. All separations were performed utilizing a mini-gel electrophoresis system (Invitrogen, USA), and methods were as described by the manufacturer. Briefly, samples of WME (10 µL) were diluted four-fold in NuPAGE LDS sample buffer, and heated for 10 min at 70°C before loading onto the gel. Each lane was loaded with 10 µL, containing 10 µg of protein, or protein standards (SeeBlue Plus2 pre-stained standards, Invitrogen, USA) before the gels were run at a constant 200 V for 35 min. The separated proteins were transferred onto nitrocellulose (NC) membranes using a semi-dry transfer apparatus (Novex XCell II, Invitrogen) at 70 A per gel for 60 min at room temperature, according to the manufacturer's instructions. After transfer, the NC membranes were incubated in PBS containing 0.5% Tween 80 and additional 0.5 M NaCl (PBS/T80/NaCl) for 30 min at room temperature on an orbital shaker (100 cycles/min) to block non-specific protein adsorption. To detect allergens, the blotted proteins were reacted with test sera, which were obtained from a pool of sera taken from nine sheep infected 12 weeks previously with *P. ovis*, as described above. Ovine antisera were diluted 1/10 in PBS/T80/NaCl, and the NC blot incubated for 1 h, with constant rocking. The blot was washed (10 min/wash) three times in PBS/T80/NaCl, before being incubated in monoclonal anti-ovine IgE (clone 2F1, 1/1000 in PBS/T80/NaCl) (10), and washed a further three times in PBS/T80/NaCl. The blot was then reacted with biotinylated goat anti-mouse Ig (Dako, 1/1000 in PBS/T80/NaCl) for 1 h, followed by a further three washes in PBS/T80/NaCl and streptavidin–horseradish peroxidase (Dako Streptavidin–HRP, 1/2000 in PBS/T80/NaCl) for 30 min. After a final three washes, peroxidase activity was revealed by chemiluminescence detection, using ECL reagent (Amersham Life Sciences, Bucks, UK) as described by the manufacturer. Control blots consisted of replacing the test ovine antisera with 1/10 dilution of sera from the same animals prior to infection.

SDS-PAGE and Western blotting of WME were also performed with antibodies to house dust mite tropomyosin, paramyosin and vitellogenin/apolipoporphin. The technique

of electrophoretic separation and Western blotting was as described above for ovine antisera, except that the primary antibodies (mouse anti-tropomyosin, paramyosin and polyclonal and monoclonal anti-(1–224) (clones 7A9 and 3F1) vitellogenin/apolipoprotein were employed at dilutions of 1/1000–1/3000, followed by goat anti-mouse-biotin (1/1000) (Dako) and Streptavidin–HRP (Dako). Depending on the sensitivity required, peroxidase activity was revealed either with diaminobenzidine (DAB)/urea/H₂O₂ substrate (Sigma Ltd, Poole, UK), or by chemiluminescent detection, as described above. Controls consisted of replacing the primary mouse antisera or monoclonal antibody with the same dilution of normal mouse serum. An experiment was also performed in order to test the specificity of the reaction to Der p 14-like homologue in *P. ovis* WME, by pre-absorption of the antibodies with recombinant Der p 14 (1–224). Monoclonal 7A9 and polyclonal anti-1–224 antibody were diluted 1/1000 in PBS/T80/NaCl containing 1 mg/mL purified recombinant Der p 14 (1–224) and incubated for 1 h at room temperature, before reacting with the blots as described above.

2-D SDS-PAGE and immunoblotting

Proteins were separated by isoelectric focusing employing the ZOOM IPGRunner System followed by SDS-PAGE employing the NuPAGE Novex 4–12% Bis-Tris ZOOM gels (Invitrogen), according to the manufacturer's instructions. Briefly, samples (100 µL of WME containing 180 µg protein), were subject to a prior '2-D Clean-Up' procedure (Ettan sample preparation kits, Amersham Biosciences Corp, San Francisco, USA), according to the manufacturer's instructions, and prepared in 155 µL of sample rehydration buffer containing 20 mM DTT and ampholytes (final concentration 0.2–2%). The samples were applied to IEF pH 3–10 strips, before subjecting to isoelectric focusing for 4 h at 500 V. Following separation, the IEF strips were pre-incubated for 15 min in reducing buffer (NuPAGE, Invitrogen), and then in 12 mM alkylating solution at room temperature for 15 min, before performing the two-dimensional separation on the Bis-Tris Zoom gels. The resultant gels were either stained for protein with Simply Blue stain (Novex, Invitrogen), or subjected to protein transfer and Western blotting as described above for 1-D SDS-PAGE gels. Immunoblotting was performed with a pool of sheep serum taken from nine sheep infected 12 weeks previously with *P. ovis*, and mouse and rabbit antibodies to the house dust mite allergens were as described above for 1-D SDS-PAGE.

Maldi-ToF mass spectrometry

Spots of interest were excised from 2-D gels using spot-picker pens (either 1.5 mm or 3.0 mm diameter.). Spots were

destained and digested overnight with trypsin (Porcine trypsin, Promega) at 37°C. Digests were analysed on a Voyager DE-PRO Maldi-ToF mass spectrometer (Applied Biosystems), scanning the 600–5000 Dalton region. The spectra generated were mass calibrated and deisotoped. The masses were then searched against existing databases using the MASCOT search engine. A Mowse score was calculated and was $-10 \times \log(P)$, where P was the probability that the observed match was a random event. According to this analysis, scores greater than 74 indicate identity based on mass ($P < 0.05$).

RESULTS

1-D and 2-D SDS-PAGE and immune blots

Preliminary analysis of the allergens in mite WME was performed on 12% SDS-PAGE gels under reducing conditions. Western blot analyses of IgE antibodies from pooled sera at pre-infection, 2 and 12 weeks post-primary infection, and at 0, 2 and 6 weeks post-secondary challenge are shown in Figure 1. A faint high molecular weight (180 kDa) reactive band was observed 14 days after primary infection, but was more pronounced after 12 weeks of infection. Following a secondary challenge of mites, the high molecular weight allergen remained prominent. However, at 6 weeks post-secondary infection this high molecular weight allergen was labelled more strongly, and a number of lower molecular

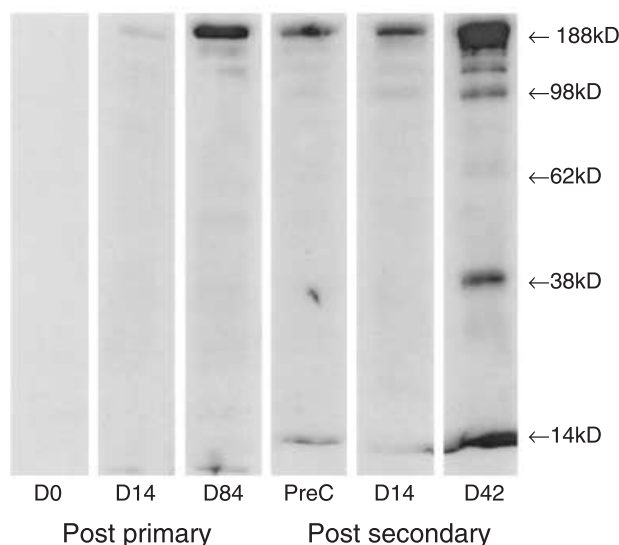


Figure 1 Reducing SDS-PAGE (12% linear tris-glycine gels)/Western blot of *P. ovis* whole mite extract, showing the IgE reactive antigens in pooled sera at pre-infection (D0), at days 14 and 84 post-primary infection, and during a secondary challenge with *P. ovis* with sera taken just prior to challenge (PreC), and 14 and 42 days post-challenge. Peroxidase reaction product detected by chemiluminescence.

weight IgE-reactive bands were also observed. For further analysis, SDS-PAGE separation was performed on 4–12% bis-tris gels under non-reducing conditions. The resultant Western blots using sera from five individual animals taken 6 weeks post-secondary challenge indicated a more complex mixture of allergens, ranging from high (180 kDa) molecular weight down to very small (< 10 kDa) IgE reactive antigens (Figure 2). A similar IgE reactive profile was observed for each animal, although the density of the staining varied, indicating quantitative, rather than qualitative, differences. Little or no reactive product was observed with control, non-infected sera (not shown). Similarly, no reaction product was observed in blots incubated without the test sera.

The WME was subjected to further analysis by 2-D SDS-PAGE. After staining for protein, numerous spots appeared (Figure 3a), and many of these reacted with IgE antibody when incubated with pooled sera from sheep 6 weeks after secondary challenge (Figure 3b). By overlaying the resultant chemiluminescent IgE hyperfilm (Amersham) image onto a scan of the protein-stained gel, 28 distinct spots or regions were selected as being IgE reactive proteins, and these were excised for Maldi-ToF analysis. This analysis resulted in 12 proteins that did not demonstrate any homology with known peptide sequences, and a further 12 spots provided sequences with very low and non-significant probability based on Mowse scores (scores of less than 74) with known proteins, with those of myosin heavy chain and *Drosophila* products predominating. Only four spots resulted in peptides that demonstrated significant homology with known peptide sequences from the database; two were homologues

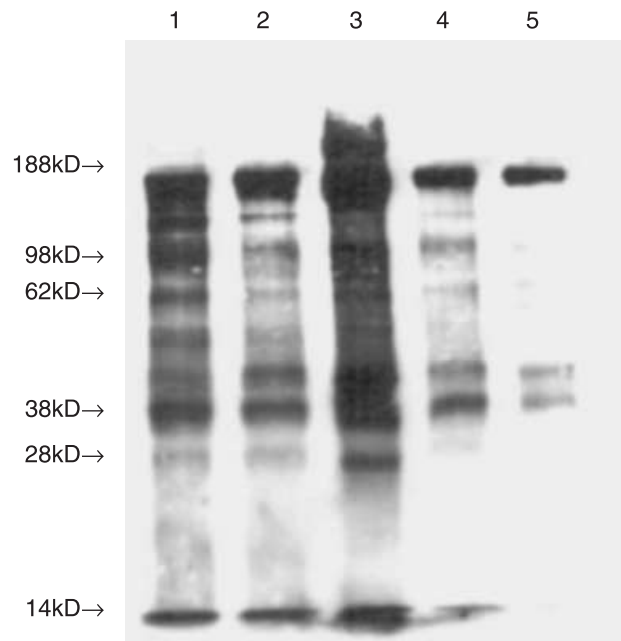


Figure 2 Non-reducing SDS-PAGE (4–12% bis-tris gels)/Western blot of whole mite extract, showing the IgE reactive bands in sera taken from five sheep 6 weeks post-secondary challenge infection with *P. ovis*. Peroxidase reaction product detected by chemiluminescence.

of a paramyosin-like allergen from the house dust mite *Dermatophagoides farinae* (Mowse scores of 82 and 151), one was a homologue of tropomyosin (Mowse score 156 for *D. pteronyssinus* and 138 for *D. farinae*), and the final

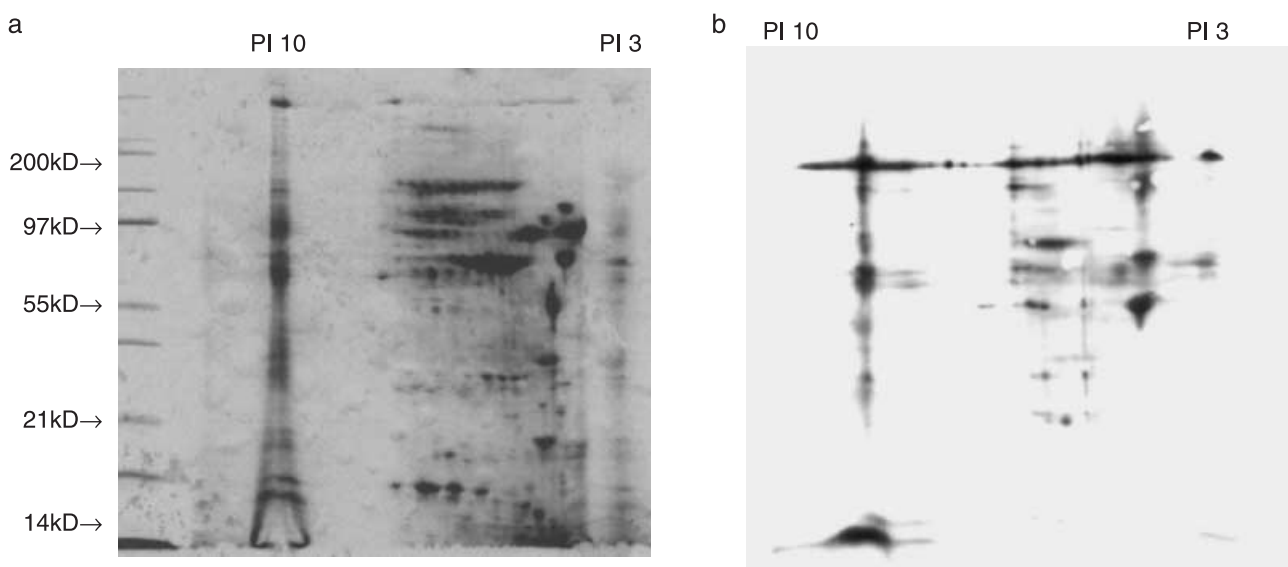


Figure 3 2-D SDS-PAGE/Western blot analysis (4–12% bis-tris gels, IEF pH 3–10) of *P. ovis* WME showing (a) distribution of proteins stained with Coomassie blue (Simply Blue, Novex) and (b), the IgE reactive proteins in immune sera following protein transfer and Western blotting. Peroxidase reaction product detected by chemiluminescence.

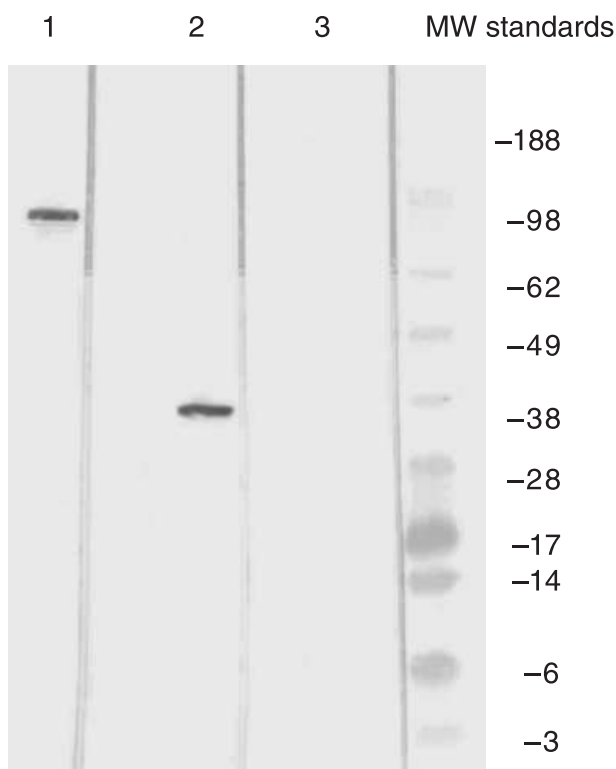


Figure 4 Western-blots of mouse anti-house dust mite tropomyosin and paramyosin (group 10 and 11 allergens, respectively), on *P. ovis* whole mite extract. Lane 1, anti-paramyosin; lane 2, anti-tropomyosin; and lane 3, normal mouse serum.

excised protein indicated significant homology with an apolipoprotein (Mowse score 119) or apolipoprotein precursor from cattle (Mowse score 116).

Western blot analysis with anti-house dust mite allergens

The Maldi-ToF analysis provided evidence that two of the three major scab mite allergens are homologues of the house dust mite allergens tropomyosin (Der p 10) and paramyosin (Der p 11), and the third allergen indicated an apolipoprotein-like protein. Since the house dust mite allergen Der p 14 has been characterized as an apolipoprotein/vitellogenin protein, antibodies generated to these group 10, 11 and 14 house dust mite allergens were investigated for their immunoreactivity on 1-D and 2-D SDS-PAGE Western blots of *P. ovis* WME. On 1-D blots, strong single bands occurred with anti-Der p 10 and Der p 11 with molecular weights of approximately 37 kDa and 98 kDa, respectively (Figure 4). Immunoblotting with polyclonal antibody to Der p 14 (1–224) resulted in several high molecular weight bands, with the predominant reactive band being 180 kDa, which was of identical size to the high molecular weight allergen detected with immune sera (Figure 5). This band was also strongly

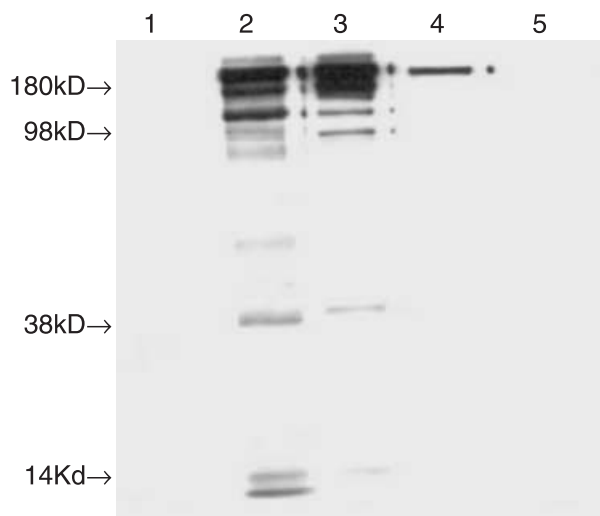
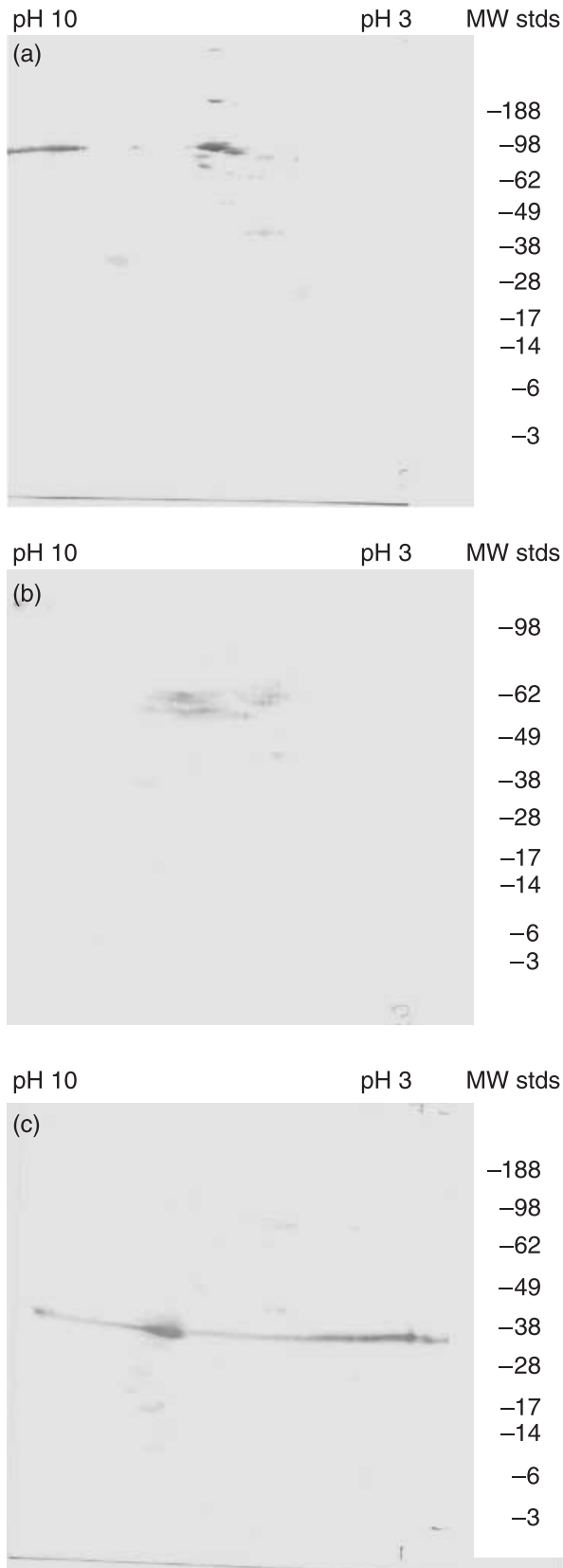


Figure 5 Western-blot of anti-house dust mite vitellogenin/apolipoprotein (group 14 allergen) on *P. ovis* WME. Lane 1, monoclonal anti-(1–224) high IgE binding peptide of group 14 allergen (clone 3F1). Lane 2, immune sera from one sheep showing IgE reactive bands for comparative purposes with lane 3, mouse serum anti-(1–224) group 14 peptide and lane 4, a second monoclonal to peptide 1–224 (clone 7A9). Lane 5, normal mouse serum control. Peroxidase reaction product detected by chemiluminescence.

recognized by monoclonal 7A9 anti-Der p 14 (1–224), and blots with this monoclonal showed a single band of 180 kDa (Figure 5). However, monoclonal 3F1 failed to react with the WME. Control blots incubated with normal mouse serum were negative (Figure 5). An experiment to confirm the specificity of the monoclonal clone 7A9 was performed by pre-incubating the polyclonal and monoclonal with excess (1 mg/mL) recombinant 1–224, prior to reacting on blots of WME and recombinant protein. Prior absorption abrogated the reaction of monoclonal 7A9 to WME or recombinant 1–224, but the polyclonal reaction on WME appeared to be unaffected, and the same profile of high molecular bands was observed compared to unabsorbed antibody.

Immunoblot analysis by 2-D SDS-PAGE/Western blotting revealed a more complex picture. Reaction with anti-Der p 11 showed three main strongly reactive spots all of an approximate molecular weight of 98 kDa; one was an elongated smear at a basic PI of 8–10 and a further two spots were demonstrated at neutral PI (Figure 6a). Two or three other, but less strongly reactive, spots were also observed. For apolipoprotein/vitellogenin, in contrast to the 1-D blots, monoclonal 7A9 anti-(1–224) Der p 14 did not react on 2-D Westerns, but the polyclonal anti-Der p 14 (1–224) showed 4–5 rather weak and indistinct spots with neutral PI, at 60–70 kDa (Figure 6b). The anti-Der p 10, however, provided



a much simpler reaction profile, and reacted strongly with a clear band spanning the PI range (3–10), at a molecular weight of 38 kDa (Figure 6c). Little or no reaction product was observed in blots incubated with the primary antibody step omitted.

DISCUSSION

Previous work has shown that infection of sheep with the sheep scab mite, *P. ovis*, results in a profound intradermal Th2 inflammatory response with the generation of IgE antibodies (2,3). The scab lesion has thus been considered as an allergic hypersensitivity reaction, and the allergens which elicit this response may be important candidates in any alternative control strategy such as vaccination. The present findings have provided firm evidence for the identification of three major scab mite allergens, which appear to generate strong IgE antibody responses during experimental primary or secondary challenge infections of sheep. These allergens, on the basis of Maldi-ToF trypsin digest analysis and reactivity with a panel of monoclonal and polyclonal antibodies, have now been identified as homologues of the Group 10, 11 and 14 house dust mite allergens.

House dust mite allergens have been extensively studied, since antigens derived from these mites are a major source of allergy in the Western world (11,12). In addition to the two major species of house dust mites, *D. pteronyssinus* and *D. farinae*, *Euroglyphus maynei* in temperate regions and *B. tropicalis* in tropical/subtropical regions may also be important as sources of allergens. Immunoblotting studies have shown that there is considerable complexity in the molecular weight range of allergens, but it is clear that there are several immunodominant specificities that span the different mite species. Many of these have now been identified, and have been grouped in order of chronological discovery and/or biochemical characteristics. One of the major house dust mite allergens is the cysteine protease and group 1 allergen, Der p 1, and previous work has shown a homologue for this allergen in *P. ovis* termed Pso o 1 (6). The expressed recombinant product of *pso o 1* was recognized by IgG and IgE antibody in immune serum from sheep, and the inferred amino acid sequence showed 53% identity and 66% similarity to Der p 1 and 53% identity and 68% similarity to Eur m 1. Further evidence for the similarity of house dust and scab mite allergens has come from an EST survey of gene

Figure 6 2-D SDS-PAGE/Western blot analysis of the house dust mite homologues in *P. ovis* whole mite extract. (a) Mouse anti-house dust mite paramyosin (group 11 allergens); (b) mouse anti-(1–224) peptide of vitellogenin/apolipoprotein (group 14) allergens; and (c), mouse anti-tropomyosin (group 10) allergens. Reaction product developed with DAB/urea/H₂O₂ substrate.

expression for *P. ovis*, where the Cluster analysis and database interrogation with the resultant nucleotide sequences derived from 507 randomly selected cDNAs, resulted in nine putative allergens with homologues in the house dust mite species (5). These included not only Group 1 allergens, but also those represented in Groups 2, 7, 10, 11 and 13. The present study has confirmed that allergen homologues to the house dust mite group 10 and 11 allergens are not only present in *P. ovis*, but generate IgE antibody responses during infection.

The group 10 house dust mite allergen has been identified as tropomyosin, and is highly conserved with approximately 75% sequence identity with other arthropod tropomyosins, with high immunological cross-reactivity to shellfish and other invertebrate tropomyosins (13). These proteins are highly allergenic and hence have been termed pan allergens, and are responsible for a significant proportion of house dust mite and shellfish allergies. The purified natural protein has a molecular weight of 37 kDa (14), and the result of the 1-D and 2-D immunoblotting with anti-Der p 10 demonstrated a similar molecular weight (38 kDa) for the scab mite tropomyosin homologue. These results, together with the high Maldi-ToF peptide homology between the sheep scab mite and the house dust mites *D. pteronyssinus* and *D. farinae* further indicate the highly conserved nature of this protein, with the *P. ovis* tropomyosin being very similar in terms of allergenicity and structure to the other mite tropomyosins.

Paramyosin, the group 11 house dust mite allergen, is a structural muscle protein of invertebrates and, like tropomyosin, is a major allergen of house dust mite allergy (11). Western blot studies of sera from house dust mite allergic individuals have shown that there is a high frequency IgE binding protein of 90–100 kDa, which is most likely to be paramyosin. The Maldi-ToF analysis combined with the immunoblotting results with anti-Der p 11 have confirmed the presence of this allergen in the sheep scab mite. On 1-D SDS-PAGE the anti-Der p 11 produced a single 98 kDa band, but the 2-D SDS-PAGE was more complex, with three or more reactive smears and spots. Since most of these reactive spots were in the region of 98 kDa, it is unlikely that they represent major degradation forms (15), but rather post-translational modifications such as glycosylation, or possibly polymorphisms, since at least three isoforms of Der f 11 have been previously identified (16). From the 2-D immunoblots with immune serum and subsequent Maldi-ToF analysis, only one of these paramyosin spots appeared to be recognized by ovine IgE antibody, suggesting that the major IgE epitopes against the scab mite paramyosin may be confined to this immunodominant form. If confirmed, this would be in contrast to the human IgE responses to the analogous house dust mite allergen, where the major IgE epitopes are scattered throughout the molecule (17).

Earlier studies on the development of the IgE antibody response to scab mite antigens indicated that immunodominant antigens of > 100 kDa and < 15 kDa were most consistent in stimulating IgG and IgE antibody responses (3). In the present study, we have shown that the most immunodominant allergen appears to be a high (approximately 180 kDa) molecular weight antigen which is recognized early during a primary infection, and is particularly prominent in animals undergoing a secondary challenge infection. Peptide sequences from Maldi-ToF analysis indicated a 'lipoprotein-like molecule', and subsequent immunoreactions on 1-D blots provided good evidence that the 180 kDa is the *P. ovis* homologue of a house dust mite allergen initially known as Mag 3 or M-177 high frequency IgE binding molecule (18), and now known as Der p 14 (12). This protein has been characterized as apolipoprotein/vitellogenin because of its dual characteristics of lipid binding and similarity to the vitellogenins (9). Der p 14 is highly sensitive to proteinase degradation (18), which may account for the multiple bands observed with the polyclonal antibody on 1-D blots in the present study, and breakdown of this large protein may also have resulted in the complex series of lower molecular weight spots observed in the 2-D blots. Nevertheless, one of the monoclonals (7A9) generated to the peptide 1–224 fragment of Der p 14 reacted strongly to a single 180 kDa band on a 1-D blot of *P. ovis* WME, which is similar to the molecular weight of Der p 14, estimated at 177 kDa (18). The other monoclonal (3F1) failed to react with the *Psoroptes* WME, however, suggesting that there are also epitope differences between the Der p 14 and the sheep scab mite homologue. The positive immunoreaction with monoclonal 7A9 was abrogated by pre-absorption with the recombinant protein, although pre-absorption failed to prevent the polyclonal from binding the *P. ovis* homologue, which was probably a result of the extended or multi-epitope binding properties of this antiserum. Peptide fragments of Der p 14, including Mag 1, have been shown to have high reactivity in IgE binding and T cell proliferation assays (9), and may act as an 'allergic adjuvant', enhancing IgE responses to itself and to other potential allergens. Whether the sheep scab mite homologue for this allergen acts in a similar manner will require further study, but it may be relevant to the lesion development that IgE antibodies to this protein appear to be generated within a few weeks of infection and that this is the first allergen that can be detected by Western blotting. This suggests an IgE-reactive immunodominance for the Der p 14 homologue, although in view of the known difficulties in detecting IgE anti-Der p 1 by Western blots (19), interpretation of the sequence and magnitude of the responses to the various allergens of the scab mite requires further study. For example, IgE binding epitopes that rely on tertiary structure may lose antigenicity and be

poorly detected by blotting, and ELISA studies with purified or recombinant proteins are required.

The 1-D Western blot analysis indicated that the three allergens identified represent immunodominant allergens of the scab mite. Nevertheless, the complexity of the IgE immunoreactions on 2-D blots suggests that there are several further allergens that are generated during infection, and studies are now ongoing to identify these. Previous work has demonstrated that *P. ovis* infections induce strong natural immunity (2), and the identified allergens may therefore represent important candidates for vaccination (20). In particular, evidence from our current studies suggests that the mite may induce allergic responses for their own benefit, such as for feeding or by inducing a microenvironment conducive for their maintenance (Huntley *et al.*, in press, Veterinary Parasitology). The prior generation of a predominant IgG antibody response by vaccination may thus inhibit or block the induction or action of IgE antibodies, and reduce these pro-inflammatory allergic reactions. Purification of sufficient quantities of native allergens for a commercial vaccine is not possible, and recombinant technology will be employed to generate recombinant tropomyosin, paramyosin and apolipophorin/vitellogenin. Perhaps of relevance is that the recombinant form of paramyosin from schistosomes has been shown to provide encouraging results in vaccination to this parasite in mice and water buffaloes (21); whether such proteins will be effective as a vaccine for the scab mite remains to be determined.

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