

Cutaneous and systemic responses during primary and challenge infestations of sheep with the sheep scab mite, *Psoroptes ovis*

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SUMMARY

Lesional area was estimated and lesional mast cells and eosinophils were counted during primary and challenge infestations of sheep with Psoroptes ovis. In addition, circulating basophils and eosinophils and serum P. ovis-specific immunoglobulin IgE antibody were quantified. Expansion of lesional area was significantly less and serum IgE titres significantly greater in challenge than primary infestations. Lesional mast cell hyperplasia and massive eosinophil infiltration accompanied by raised titres of P. ovis antigen-specific IgE antibody were compatible with an IgE-mediated Type-1 allergic reaction, while detection of lesional basophils was suggestive of cutaneous basophil hypersensitivity. The temporal pattern of lesional mastocytosis and eosinophil infiltration and the role of these cells and serum IgE in the immune response is discussed.

Keywords *Psoroptes ovis*, immediate hypersensitivity, IgE, mast cells, eosinophils

INTRODUCTION

The astigmatid mite *Psoroptes ovis* is an obligate, nonburrowing, ectoparasite. It causes sheep scab, a cutaneous disease characterized by pruritus and exuberant scab formation. High morbidity and intense pruritus accompanied by damage to fleece and hide, loss of condition and occasional mortality make this a disease of considerable economic and welfare importance (Kirkwood 1980, 1985, Sargison *et al.* 1995). Current control measures employing organophosphate compounds and synthetic pyrethroids raise human health and ecological concerns (Stephens *et al.* 1995, Gasser *et al.* 1997). Furthermore, the development of strains resistant to these chemicals has been reported (Synge *et al.* 1995, Clark *et al.* 1996). Therefore acceptable alternative methods of control need to be developed.

Studies of primary and challenge infestations of calves (Stromberg & Fisher 1986) have demonstrated a significant reduction in fecundity and population density of *P. ovis* mites, as well as retarded lesional expansion in challenge infestations. This suggests that cattle develop a significant protective immunity to *P. ovis* infestations. Similar studies in sheep have not been reported.

P. ovis antigen-specific immunoglobulin IgG but not IgE antibody titres have been monitored in primary infestations of sheep (O'Brien 1992, Bates 1997). However, lesional histopathology of naturally occurring infestations of sheep has revealed a pronounced eosinophil infiltrate (Rovere & Nunez 1977, Rosa & Moschini 1988) and sodium dodecyl sulphate-polyacrylamide gel electrophoresis/Western blot studies (Matthes *et al.* 1996) have demonstrated the presence of *P. ovis* antigen-specific IgE antibody in sera from infested sheep and deer. These observations are consistent with the participation of immediate hypersensitivity in the immunopathogenesis of cutaneous lesions but give no indication of its role in protective immunity.

Temporal correlation between mucosal mast cell numbers and worm expulsion has provided evidence for

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the involvement of IgE-mediated Type-1 allergic reactions in resistance to certain intestinal nematodes (Woodbury *et al.* 1984). In this study, we report observations made on the temporal pattern of lesional mast cell and eosinophil numbers and of lesional growth during primary and challenge infestations of sheep. In addition, we present the results of monitoring serum *P. ovis* antigen-specific immunoglobulin IgE antibody titres in the course of these infestations. Accompanying alterations in circulating eosinophils and basophils are also reported.

MATERIALS AND METHODS

Animals

Primary infestation (group 1)

Ten *P. ovis* naïve Suffolk cross sheep aged one to two years were infested on the withers with 25–50 ovigerous *P. ovis* mites which were removed from donor sheep with a mounted needle and selected by examination under a dissecting microscope with overhead lighting (American Optical, Buffalo, NY, USA, 40× magnification). In each animal, lesional area was measured and blood samples were taken for routine haematology and serology at weekly intervals for 14 weeks postinfestation (p.i.). After injection of a local anaesthetic (Lignol, Arnolds Veterinary Products, Harlescott, Shrewsbury, UK) 6 mm punch biopsies were collected from the advancing border of the lesion at 0, 3, 6, 9 and 12 weeks p.i. The infestation was terminated by injection of an endecticide (Ivomec, Merial Animal Health, Harlow, Essex, UK, 200 µg/kg) at 12 and 13 weeks p.i. One animal collapsed with epileptiform convulsions 6 weeks p.i. and was excluded from the trial.

Secondary (challenge) infestation (group 2) and controls (group 3)

Five of the sheep from group 1 were maintained mite-free for 11 weeks post-treatment and then were infested with 25–50 ovigerous *P. ovis* mites (group 2). In addition, five *P. ovis* naïve, control sheep (Suffolk cross and aged 1–2 years), were infested with 25–50 ovigerous *P. ovis* mites of the same strain (group 3). Lesional area, routine haematology and serology were monitored in both groups, as described above, for 8 weeks p.i. Biopsies were collected from both groups at 0, 3 and 6 weeks p.i.

Lesional area

The method described by Guillot (1981) was modified and used to estimate lesional area. Briefly, the lesional shape and dimensions were mapped onto graph paper and used to determine the lesional area.

Histopathology

Biopsies were fixed in 4% paraformaldehyde in phosphate buffered saline pH 7.4 for 6 h (Newlands *et al.* 1984), then stored in 70% ethanol at 4°C (Miller *et al.* 1983), processed and embedded in paraffin wax. Serial 5 µm tissue sections were cut. Mast cells were stained with toluidine blue 0.5% in 0.5 N HCl, pH 0.5 (Enerback 1966) for 18 h. Eosinophils were stained with Lendrum's carbol chromotrope solution (Sigma, St Louis, MO, USA) for 1 h (Lendrum 1944) and counterstained for 10 s with haematoxylin. Dermal mast cells and eosinophils in a minimum of 40 successive graticule fields on each of two semiserial sections were counted using an Olympus BX 50 microscope at 400× magnification. Intravascular cells were excluded from the count.

Haematology

Samples were collected into heparinized tubes (Becton Dickinson, Franklin Lakes, NJ, USA) by jugular venipuncture. Total white blood cell count was determined with a Baker 9110 Haematology Analyser (Biochem. Pharma. Inc., Allentown, PA, USA). The differential count was estimated manually after staining a blood smear with a modified Wright's stain.

Assay of IgE

In order to assay ovine IgE antibody, serum samples (100 µl) were heated at 56°C for 30 min and then precipitated in 30% and 50% saturated ammonium sulphate (Kooyman *et al.* 1997). The final precipitate was dissolved in 200 µl phosphate buffered saline (PBS) containing 0.5% Tween 80 and 0.5 M NaCl (PBS/T80). Micro-enzyme-linked immunosorbent assay plates (M129B, Dynex Technologies, Ashford, Middlesex, UK) were incubated for 1 h at room temperature with (50 µl/well) *P. cuniculi* whole-mite antigen (10 µg/ml) in carbonate/bicarbonate buffer pH 9.6. Wells were washed twice with PBS/Tween 20, before adding 50 µl of preprepared sera. Plates were then sequentially incubated with 50 µl of a 1 : 10 dilution in PBS/T80 of mouse anti-ovine IgE monoclonal antibody (IE7, Kooyman *et al.* 1997), 50 µl (1 : 1000 dilution in PBS/T80) biotinylated goat antimouse Ig conjugate (Dako, Ely, Cambs, UK) and 50 µl streptavidin (Dako). Each incubation was for 1 h at room temperature and plates were washed six times in PBS/Tween 20 after each incubation. Colour reaction was developed by incubating with 50 µl 3,3',5,5'-tetramethylbenzidine (TMB) substrate for 30 min at room temperature and stopped by the addition of 50 µl 0.18 M H₂SO₄ (Voller *et al.* 1979). The colour reaction at OD 450 nm was measured in a Labsystems iEMF Reader (Labsystems Life Sciences International,

Hampshire, UK). In order to compare results between assays, aliquots from a pool of serum containing IgE antibody were stored at -20°C , serum from a fresh aliquot was included on each plate and the OD 450 of unknown samples were adjusted relative to this. In practice, between test variability was minimal.

Statistical analysis

The results are expressed graphically as mean $\pm$ SEM. Differences between the groups were determined by analysis of covariance using a Genstat 5 Release 4.1 programme (Lawes Agricultural Trust; Numerical Algorithms Group Ltd, Oxford, UK). Values of $P < 0.05$ were considered significant.

Image capture

Images were acquired with a JVC TK-C1381 colour video camera attached to a Leica Lablux S conventional compound microscope (Leica Microsystems UK Ltd, Milton Keynes, UK). Live 24-bit super-VHS colour PAL video was digitized using a Power Macintosh 7100/80 fitted with an AV digitiser (Apple Computer, CA, USA). Acquisition, scaling and annotation of images were performed using Enhanced Image Acquisition v1.0 (Jeremy Brown, Department of Veterinary Clinical Studies, The University of Edinburgh, Edinburgh, UK) running under Object-Image1.62p6. Object-Image is a public domain software package, based on NIH Image (Rasband & Bright 1995), developed by Norbert Vischer (Universiteit van Amsterdam, Amsterdam, The Netherlands) and is available from the internet by anonymous FTP (for details, see <http://simon.bio.uva.nl/object-image.html>).

RESULTS

Lesional area

Logarithmic transformation of area ($\log_{10} + 1 \text{ cm}^2$) revealed a rapid, exponential, increase in lesional area on sheep in group 1 and group 3 and restricted lesional growth in group 2 sheep (Figure 1). After 1 week the mean lesional area in challenged (group 2) sheep was 2 cm^2 but no further significant increase in area occurred. The lesional area in challenged sheep was significantly less than in the other sheep at 2 weeks p.i. ($P < 0.01$) and from 3 to 8 weeks p.i. ($P < 0.001$). Clinical development of the lesion was more rapid following challenge than primary infestations, with a discrete area of inflammation and exudation evident at the site of infestation within 24 h and crust formation within 48 h of infestation.

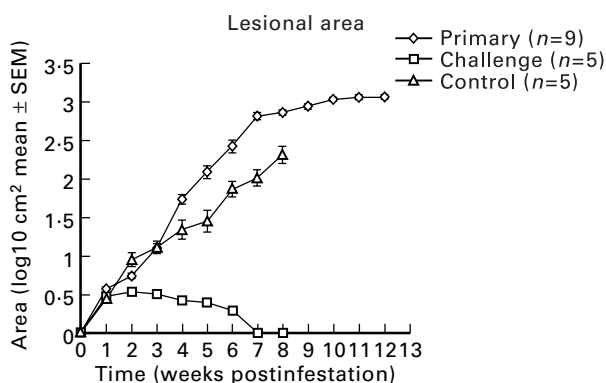


Figure 1 Lesional area.

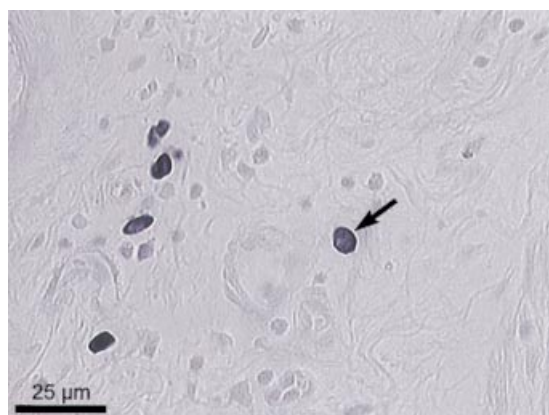


Figure 2 Normal skin. Mast cells stained with toluidine blue (mast cells, arrow, appear dark against the paler background).

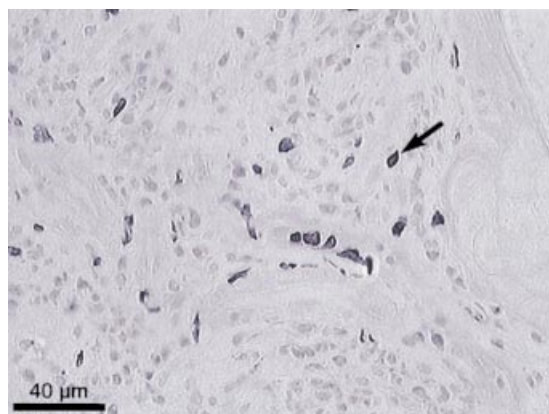


Figure 3 Lesional skin demonstrating mast cell hyperplasia 3 weeks postinfestation. Toluidine blue stain (arrow indicates typical mast cell).

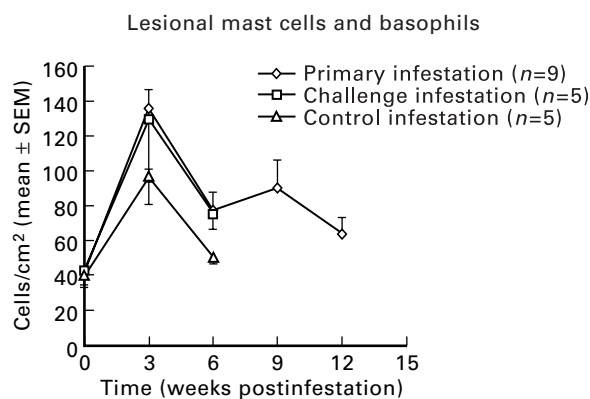


Figure 4 Lesional mast cells and basophils.

Histopathology

Marked dermal mast cell hyperplasia occurred (Figures 2 and 3) and followed a similar temporal pattern in all groups with no statistically significant differences in numbers between the groups. Numbers were maximal 3 weeks p.i. and decreased significantly from 3–6 weeks p.i. (Figure 4). In all groups, at all stages postinfestation, a high portion of cells were partially degranulated particularly in the upper dermis. Basophils, identified as small, round cells with large granules, were not seen in normal skin but were detected in all groups postinfestation and were present in the dermis and overlying scab.

A massive infiltration of eosinophils occurred. The cells were distributed throughout the dermis with increased numbers, exhibiting marked degranulation, in the upper dermis (Figure 5). Subcorneal eosinophilic pustules (Figure 6) were frequently observed in all groups and numerous eosinophils were present in the overlying scab. At 3 weeks p.i., the mean count (Figure 7) was similar in all groups but

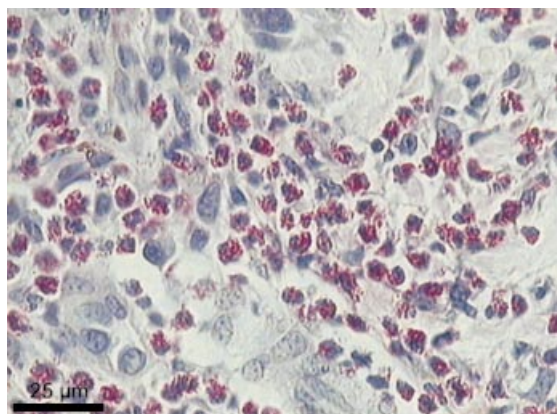


Figure 5 Lesional skin with massive infiltrate of eosinophils 9 weeks postinfestation. Carbol chromotrope and haematoxylin stain.

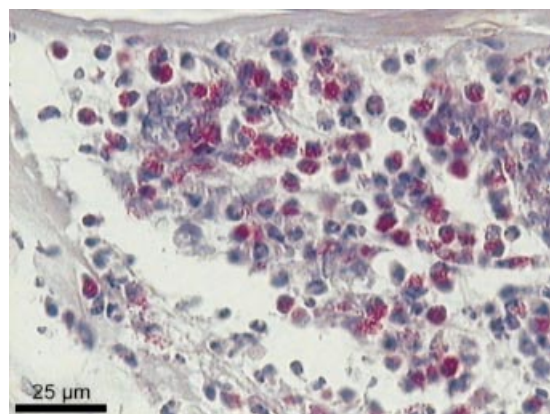


Figure 6 Lesional skin 9 weeks postinfestation showing a subcorneal eosinophilic pustule.

by 6 weeks p.i., the count in group 2 sheep had fallen and was significantly lower than counts in group 1 ($P < 0.001$) and group 3 ($P < 0.05$) sheep which had continued to rise. Numbers of eosinophils peaked at 9 weeks p.i. in group 1 animals and subsequently fell.

Haematology

Prior to infestation the mean eosinophil count (Figure 8) of the controls (group 3) was initially higher than that of the primary and challenge sheep (groups 1 and 2, respectively). However, after primary infestation, a similar increase in mean count was recorded in groups 1 and 3. In group 1, the count peaked at 11 weeks p.i. and subsequently declined. All primary and control infestation sheep (groups 1 and 3, respectively) but only one of the challenge infestation sheep (group 2) developed an absolute eosinophilia ($> 1 \times 10^9/l$). Although the mean count in the challenged

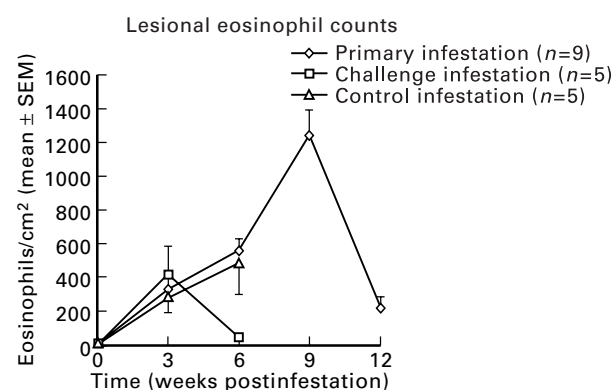


Figure 7 Lesional eosinophil counts.

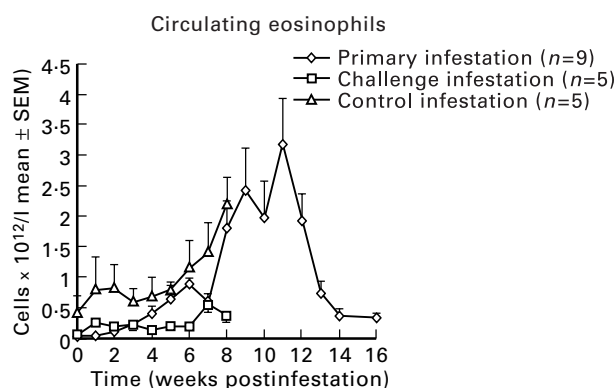


Figure 8 Circulating eosinophils.

sheep was consistently lower than in the other sheep, the difference was significant ($P < 0.01$) only at 8 weeks p.i.

The basophil count (Figure 9) was erratic and rose earlier in the control and challenge infestation groups compared to the primary infestation group. An absolute basophilia ($> 0.3 \times 10^9/l$) was detected in four of the nine sheep in primary infestation group, two of five sheep in the control infestation group and one of five sheep in the challenge infestation group. No statistically significant differences were detected between the three groups of sheep.

Serology

After primary infestation, the increase in IgE antibody titre (Figure 10) observed in group 3 paralleled that in group 1 with the initial rise in titre detected 6–7 weeks p.i. The peak titre occurred 13 weeks p.i. in group 1 sheep and then declined. In challenged sheep, there was a rapid anamnestic response and except at 5 weeks p.i. the IgE antibody titre in these sheep was significantly higher ($P < 0.03$, $P < 0.01$) than in the other sheep from 1–8 weeks p.i.

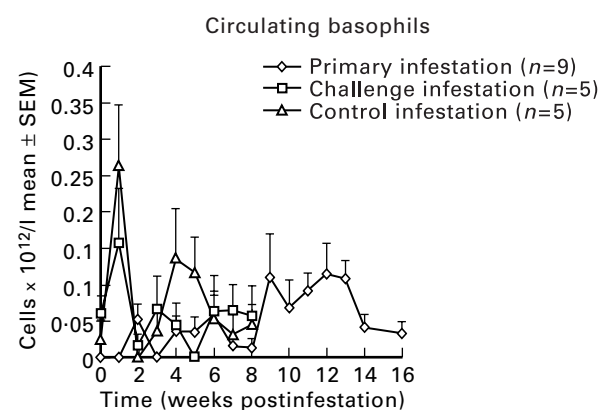


Figure 9 Circulating basophils.

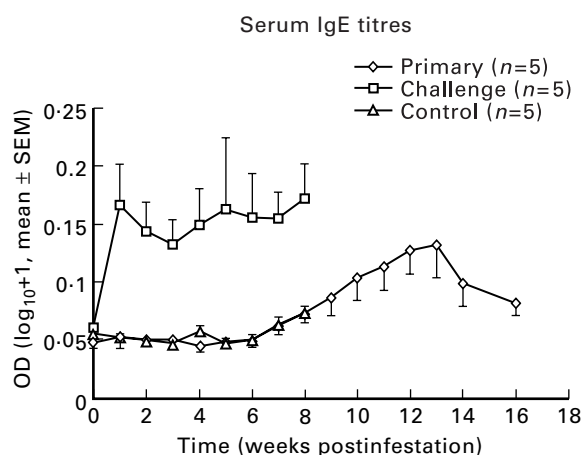


Figure 10 Serum IgE titres.

DISCUSSION

The observation, in this study, of a significant reduction in lesional growth following challenge infestation demonstrated that mite products induce the development of a substantial protective immunity to *P. ovis* in sheep. Retarded lesional growth in a similar challenge infestations of cattle (Stromberg & Fisher 1986) and rabbits (Uhlir 1991) and following infestation of calves (Pruett *et al.* 1998) and rabbits (Uhlir 1992) vaccinated, respectively, with *P. ovis* and *P. cuniculi* antigens has also been reported. Currently, the mechanism mediating protection is unknown. However, observations made in challenged cattle suggest that protective immunity impairs mite fecundity (Stromberg & Fisher 1986, Guillot & Stromberg 1987). Increased grooming, stimulated by pruritus resulting from an immediate hypersensitivity reaction to mites, may also contribute to the reduction in mite population on challenged cattle (Pruett *et al.* 1998) but, in the present study, no evidence of increased grooming or self-trauma was observed in challenged sheep.

Lesional histopathology of primary and challenge infestations, documented in this study, indicated that mite products provoked a profound cutaneous inflammatory response. This was characterized by an intense eosinophil infiltrate and marked mast cell hyperplasia and accompanied by variable circulating eosinophilia. Similar observations have been reported following experimental infestations of calves (Stromberg & Fisher 1986, Stromberg & Guillot 1989). Serology indicated that mite products stimulated the generation of *P. ovis* antigen-specific serum IgE antibody. Its detection relatively late in the course of primary infestation may reflect the sensitivity of the assay. However, as a result of the selective uptake of IgE by mast cells, its functional activity in tissue may precede its detection in serum. These cellular changes and accompanying *P. ovis*

antigen-specific serum IgE antibody provide strong evidence that *P. ovis* products induce immediate hypersensitivity reactions. The detection of lesional basophils and circulating basophilia, not reported previously, suggests that they also provoke cutaneous basophil hypersensitivity reactions.

The immediate hypersensitivity response appears to dominate the immunopathogenesis of cutaneous lesions but its contribution and that of basophil hypersensitivity to protective immunity is uncertain. Earlier studies of experimental infestations of naïve cattle (Pruett *et al.* 1986) and sheep (Bates 1997) have suggested that, after an initial 'lag' phase with minimal increase in mite population and lesional area, the development of immediate hypersensitivity to mite antigens promoted a phase of accelerated expansion of the mite population and therefore lesional area. However, in the present study, the increase in lesional area of primary infestations throughout the first 7 weeks p.i. was exponential with no evidence of either a 'lag' phase or a phase of accelerated lesional expansion attributable to the development of an immediate hypersensitivity response. However, in these studies, the marked restriction of lesional area associated with the pronounced IgE response of challenged sheep may indicate the involvement of immediate hypersensitivity in the protective response.

The potential contribution of cells associated with immediate hypersensitivity and basophil hypersensitivity to protection is suggested by studies of resistance to ticks. These have demonstrated reduced resistance in guinea-pigs administered antibasophil or antieosinophil antibodies (Brown *et al.* 1982) and the failure of mast cell-deficient mice to acquire resistance to infestation with ticks (Matsuda *et al.* 1985). However, studies of the contribution of mast cells and eosinophils to resistance in nematode infections present conflicting data (Rothwell 1989, 1996b, Miller 1996a) and it is clear that while mast cells are pivotal in resistance to certain nematodes they may increase the fecundity of others (Miller 1996a). Nevertheless, histochemical studies of mast cells, despite their limitations (Rothwell 1989), have demonstrated a temporal association between the kinetics of histologically detectable mast cells, their functional activity and worm expulsion and have provided evidence for the protective role of immediate hypersensitivity in certain intestinal nematode infections (Woodbury *et al.* 1984, Tuoy *et al.* 1990). The significant increase in histologically detectable mast cells observed 3 weeks p.i. was followed by a significant decline in detectable cells, probably as a result of further, extensive degranulation (Stromberg & Guillot 1989). These significant alterations in mast cell numbers during the first 6 weeks after primary infestation were not accompanied by any detectable change in the pattern of lesional expansion.

Although accurate estimation of the mite population in the course of infestations is difficult and was not attempted in this present study, studies in cattle (Stromberg & Fisher 1986, Stromberg *et al.* 1986) and sheep (P. Bates, personal communication) suggest that lesional expansion may be associated with increases in the mite population. Therefore, failure to document any change in the rate of lesional expansion suggests that, in primary infestations, IgE-mediated type 1 allergic reactions do not cause an immediate reduction or increase in the mite population. However, Stromberg & Fisher (1986) found that fecundity of mites placed on *P. ovis*-naïve calves was initially high and subsequently declined, whereas in challenge infestations the fecundity of mites was consistently low. In primary infestations, this would result in a delayed fall in mite numbers and would be consistent with the observation in this present study of a reduction in lesional expansion from 7 weeks p.i. In challenge infestations, early impairment of mite fecundity, if severe enough, may result in failure of the challenge population to expand and may explain the apparent inability (noted in this present study) of challenge infestations to become established. Although, there is currently no evidence that mast cell mediators have a direct influence on survival or fecundity of *P. ovis* mites, they may increase epidermal permeability and facilitate translocation of plasma proteins, including complement and mite antigen-specific immunoglobulins which may exert antimite effects. Mast cell mediators may also influence secretion from adnexal glands and contribute to accompanying changes in the cutaneous microclimate which may be inimical to mite survival.

Eosinophils dominated the lesional infiltrate and the transepidermal efflux of cells observed in this present investigation. Both eosinophils and basophils have been detected in the digestive system of ticks and granules from both cells have been demonstrated in damaged midgut cells (Voss-McCowan 1996). Eosinophils have also been observed in the midgut of *P. ovis* mites obtained from infested sheep (Matthieson 1995) and may together with ingested basophils cause similar damage in *P. ovis* mites. The rapid reduction in mean eosinophil count observed 9 weeks postinfestation of naïve animals probably paralleled a decline in mite numbers and products at the periphery of the lesion. However, while the lesional eosinophil count fell, the circulating eosinophil count continued to rise. This may be explained by reduced transepidermal loss of cells combined with a delay in normalization of progenitor cell activity. A similar fall in lesional eosinophil numbers occurred 3 weeks after challenge infestation but was not accompanied by an increase in circulating eosinophils. This may reflect differences in magnitude of the mite population and therefore the stimulus provoking eosinophil production.

In this study, a significant elevation in *P. ovis* antigen-specific IgE titre and reduction in lesional area in challenge compared to primary infestations were detected within 3 weeks of infestation. However, at this time, mast cell and eosinophil numbers in primary and challenge infestations were not significantly different. This may indicate that, in addition to mediating mast cell degranulation, *P. ovis* antigen-specific IgE has a further direct or indirect influence on mite survival or fecundity.

This investigation has provided evidence for the development of a significant protective immunity following primary infestations of sheep with *P. ovis* and for the involvement of IgE-mediated type 1 allergic reactions and cutaneous basophil hypersensitivity in the immunopathogenesis of lesions. However, the contribution of these hypersensitivity responses to the protective immunity is unclear. Transepidermal efflux of antigen-specific immunoglobulins, particularly IgE and of mast cells, eosinophils and basophils and their mediators, may influence mite survival and modulate mite fecundity but further investigations to elucidate their role are required.

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