



Immune responses to *Staphylococcus aureus* and *Psoroptes ovis* in sheep infected with *P. ovis* — the sheep scab mite

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Abstract

In sheep, lesions caused by *Psoroptes ovis*, the sheep scab mite, may become colonized by *Staphylococcus aureus*. The present study compares clinical signs, lesional area and the immune response to *P. ovis* and *S. aureus* in *P. ovis*-infested sheep with and without secondary *S. aureus* infection. No differences were detected in the clinical signs or lesional areas in the *S. aureus*-positive and -negative sheep. However, 6 weeks after infestation an IgG but not IgE isotype antibody response to *S. aureus* was detected in the *S. aureus*-positive but not the *S. aureus*-negative group of sheep. This response targeted *S. aureus* antigens with molecular weights of approximately 36, 38, 50 and 65 kDa. In addition, 6 weeks after infestation an IgE response to *P. ovis* was detected in the *S. aureus*-positive but not the *S. aureus*-negative group of sheep.

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1. Introduction

Sheep scab is typically a severe, debilitating, exudative dermatitis accompanied by exuberant crust formation

and intense pruritus. It is caused by the highly contagious astigmatid mite *Psoroptes ovis*. Since 1992 when dipping of sheep to control the disease ceased to be compulsory, the infestation has become endemic in both hill and lowland sheep in all areas of the British Isles (van den Broek and Huntley, 2003). As the disease is accompanied by severe pruritus, significant self-inflicted damage to the fleece and skin, loss of condition and occasionally death, it is of major welfare and economic concern to the UK sheep industry (Sargison, 1995).

Abbreviations: WME, whole-mite extract; CI, confidence interval

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Earlier studies have demonstrated that *P. ovis* elicits an intense early innate response characterised by lesional eosinophilia and that later this response is augmented by the development of immediate, late phase and delayed hypersensitivity responses (van den Broek and Huntley, 2003; van den Broek et al., 2000). Recently, it has been suggested that the intense immunoinflammatory response caused by *P. ovis* may be exacerbated by bacteria (Hamilton et al., 2003).

Staphylococcus aureus has been isolated with increased frequency and in greater numbers from lesional skin of human patients with atopic dermatitis than from the skin of normal subjects (Hauser et al., 1985; Matsui and Nishikawa, 2003). It is accepted that *S. aureus* is a source of potent superantigens that exacerbate immunoinflammatory responses in human atopic dermatitis (Novak et al., 2003; Taskapan and Kumar, 2000) and may augment allergen-specific IgE synthesis by atopic patients (Hofer et al., 1999) and mice (Matsui and Nishikawa, 2003). *S. aureus* has been isolated from normal skin/fleece of sheep (Chin and Watts, 1992; Meyer et al., 2001) and from lesional skin of sheep infested with *P. ovis* (Billingsley, 2003). However, although *S. aureus* is a common opportunist pathogen and has been identified as the cause of ovine staphylococcal dermatitis (Scott et al., 1980; Scott and Murphy, 1997; Synge et al., 1985) and generalized staphylococcal scalded skin-like disease in lambs (Yeruham et al., 1999), the immune response of sheep to *S. aureus* infections does not appear to have been reported.

The purpose of this study was to investigate the occurrence of *S. aureus* in lesional skin of sheep infected with *P. ovis*, to determine whether the presence of *S. aureus* influenced the development of clinical lesions and to study the immune response to *S. aureus*.

2. Materials and methods

2.1. Animals

Samples were collected from 10 *P. ovis*-naive crossbred Suffolk sheep aged 1–2 years that were infested between the withers with 25–50 ovigerous *P. ovis* mites, as described previously, (van den Broek et al., 2000) as part of an ongoing research programme. This research was approved by the experiments

Committee of the Moredun Research Institute and the animals' welfare was monitored daily in accordance with guidelines agreed with the Home Office.

2.2. Bacterial counts and identification

Samples for bacterial culture were collected from seven sheep before infestation and from 10 sheep 6 weeks after infestation with *P. ovis*. Before infestation samples were collected from the dorsum and after infestation samples were collected from the advancing edge of lesions. The cup and scrub technique described earlier (Lloyd, 1984) was employed with minor changes. Briefly, the hair was clipped close to the skin with flamed scissors. Then the skin was scrubbed for 1 min with 4 ml of washing fluid (0.1% Triton 100 in 0.075 M PBS, pH 7.9) using a 2.1 cm diameter cylinder (BD Discardit YY, Huesca, Spain) and a rod (BD Discardit YY, Huesca, Spain). Subsequently, the sample was aspirated with a syringe (BD Discardit YY, Huesca, Spain), transferred into sterile tubes and shaken manually for 30 s. Serial 10-fold dilutions of the samples were made using washing fluid diluted 1:2 in distilled water. The samples were then allowed to stand for 15–30 min.

The Miles-Misra technique (Quin et al., 1993) was employed to determine bacterial counts. Briefly, inoculums of 0.02 ml of the original and diluted samples were placed on a blood agar plate (Oxoid Limited, Hants, UK) and incubated for 48 h at 37 °C under aerobic and anaerobic conditions. At 48 h the number of colony forming units (CFU) was counted.

All bacteria cultured were identified to genus level. All staphylococcal species were examined for coagulase, hyaluronidase, haemolysin and DNase production. The identity of *S. aureus* was confirmed using an API Staph system (bioMérieux, Lyon, France). *Bacillus* sp. was identified using morphological features including Gram stain and colonial characteristics (Quin et al., 1993).

2.3. Lesional area and clinical signs

At the time of infestation the sheep had no clinical evidence of cutaneous lesions. Lesions developed at the site of infestation on the withers and progressed caudally and ventrally over the dorsum and flanks. At 6 weeks after infestation the lesional area was

determined in all ten sheep — the length of the lesion along the dorsum and its width from the most ventral point on one flank across the dorsum to the most ventral point on the contralateral flank were measured and the area (cm²) was calculated by multiplying the measured length and width. The severity of the clinical signs (degree of erythema, exudation, crust formation at the advancing edge of the lesion and pruritus) were evaluated subjectively 6 weeks after infestation. Although these methods of evaluating lesional area and severity have limitations they are well established (Bates, 1999; Huntley et al., 2005; Smith et al., 2002).

2.4. Serum samples

Before and 6 weeks after infestation blood samples were taken from all 10 sheep. Blood was collected into vacutainer tubes, containing no anticoagulant (Becton Dickinson, Franklin, NJ, USA), by jugular venipuncture and the serum was harvested.

2.5. Preparation of *S. aureus* and *P. ovis* antigens

S. aureus antigens were prepared as follows. Colonies identified as *S. aureus* were harvested and subcultured on blood agar under aerobic conditions in order to obtain pure cultures. These were diluted in 10 ml of sterile distilled water and washed by vortexing for 1 min and then centrifuged at 4000 rpm for 5 min. The supernatant was discarded, 5 ml of phosphate-buffered saline (PBS 0.1 M, pH 7.4) was added and the bacteria washed again by vortexing. This procedure was repeated three times. The washed bacteria were resuspended in 4 ml of protein extracting buffer containing 125 mM ammonium bicarbonate (Sigma, Gillingham, Dorset), 5 mM of ethylenediaminetetraacetic acid (Lab FSA Supplies, Louisiana, USA) and 250 µl of Protease Inhibitor Cocktail for General Use (Sigma, Gillingham, Dorset). The sample was then vortexed for 2 min and left in ice for 1 h. To increase extraction of protein the sample was subjected to two, 20 s cycles of mechanical disruption by 0.4 mm glass beads (Qiagen, Venlo, The Netherlands) in a ribolyzer (6.5 m/s). Between each cycle the sample was cooled in ice for 10 min. The suspension obtained was centrifuged for 5 min at 14,000 rpm. The supernatant was collected and sterilized through a 0.2 µm syringe filter. An aliquot of the extract was incubated in blood agar for 48 h to confirm sterilization.

P. ovis whole-mite extract (WME) was prepared as described earlier (van den Broek et al., 2000). Mites (adults, nymphs and larvae) were collected from infested sheep and washed by vortexing in ice-cold phosphate-buffered saline (PBS, pH 7.2) for 5 min. This was followed by a wash in dodecyl sulphate and then ten washes in ice-cold PBS. The mites were then homogenised and sonicated in ice-cold PBS and the final homogenate was centrifuged at 10,000 × g for 10 min. The supernatant was harvested, aliquoted and stored at −70 °C.

The protein content of the *S. aureus* and the *P. ovis*-whole-mite extract was measured using BCATM Protein Assay Kit (Pierce Chemical Co., Rockford, USA) in accordance with the manufacturer's instructions. Protein concentration of the extracts of *S. aureus* and *P. ovis* were, 0.298 and 1 mg/ml, respectively.

2.6. SDS-PAGE and Western immunoblotting

Sodium dodecyl sulphate polyacrylamide gel electrophoresis was performed using a continuous 4–12% Bis-Tris Gel (NuPage Invitrogen System, Carlsbad, CA, USA). Each well was loaded with 4.84 µg of the *S. aureus* extract prepared with 25 µl of NuPage Sample reducing agent (Invitrogen, Carlsbad, CA, USA), and 62.5 µl of LDS Sample buffer (Invitrogen, Carlsbad, CA, USA) made up to a final volume of 250 µl with distilled water and incubated at 70 °C for 10 min. Two broad range molecular weight markers (See Blue Plus2, Novex, San Diego, USA and Magic Marker XP, Invitrogen, Carlsbad, CA, USA) were loaded into additional wells. The gels were run at 200V (Novex Minigel system, Invitrogen, CA) for 35 min in NuPAGE MES SDS Running buffer, (Invitrogen, Carlsbad, CA, USA) with added NuPAGE Antioxidant (Invitrogen, Carlsbad, CA, USA). Gels not used for immunoblotting were stained with Coomassie Blue solution (Simply Blue Safe Stain, Invitrogen, Carlsbad, CA, USA) for 2 h and washed in distilled water overnight.

The proteins were transferred from the gel to a nitrocellulose membrane (0.2 µm pore size, Schleicher & Schuell, Dassel, Germany) by a wet transfer apparatus at 30 V for 1 h using NuPAGE transfer buffer (Invitrogen, Carlsbad, CA, USA). Membranes containing the proteins were stained with 0.1% (w/v) Ponceau S in 5% (v/v) acetic acid (Sigma, Gillingham, UK) for 5 min and then cut into strips corresponding to

the lanes, washed in distilled water and followed by a final wash in PBS/0.5% (v/v) Tween 80/0.5 M NaCl (PBS/T80/NaCl). The strips with molecular weight markers were dried and the strips with the antigen were incubated in PBS/T80/NaCl for 30 min on an orbital shaker (100 cycles/min) to block non-specific protein adsorption and then probed with test sera. Antigens binding IgG antibody were identified with sera prepared at a dilution of 1/100 in PBS/T80/NaCl. Reactivity with IgE antibodies was probed with sera diluted at 1/10 in PBS/T80/NaCl. After incubation for 1 h at room temperature (RT) with constant rocking the blots were given three 5 min washes with PBS/T80/NaCl. Subsequently, the strips were incubated in either mouse anti-ovine monoclonal antibody IgG (VPM6, 1/1000 in PBS/T80/NaCl) or mouse anti-ovine monoclonal IgE (clone 2F1, 1/1000 in PBS/T80/NaCl) for 1 h. This was followed by incubation with biotinylated goat anti-mouse Ig (DAKO, Ely Cambs, UK) diluted 1/5000 in PBS/T80/NaCl for 1 h and streptavidin–horseradish peroxidase (DAKO, 1/5000 in PBS/T80/NaCl) for 30 min. Incubations with the monoclonal antibody and biotinylated goat anti-mouse Ig were at RT on an orbital shaker (100 cycles/min) and followed by three 5 min washes in PBS/T80/NaCl. The incubation with streptavidin–horseradish peroxidase was followed by an overnight wash in PBS/T80/NaCl. Peroxidase activity in the blots was detected by chemiluminescence as described by the manufacturer (ECL Plus, Amersham biosciences, Buckinghamshire, UK). Sera collected from sheep before primary and after challenge infestation with *P. ovis* were used, respectively, as negative and positive controls. In additional control blots, serum, monoclonal antibody, biotinylated goat anti-mouse Ig and streptavidin–horseradish peroxidase were replaced by wash diluent.

The sera were also tested for IgG and IgE reactivity against *P. ovis* antigens. SDS-PAGE gels with 5 µg of *P. ovis* antigen per well were run, as described above. Western blots, including positive and negative controls, were prepared as described above except that the sera were used at a dilution of 1/10.

2.7. Statistics

Data (lesional areas and bacterial counts) was logarithmically transformed ($\log_{10}$) to calculate the

geometric mean and confidence interval (CI). Differences between the groups were determined by analysis of raw data with the Mann–Whitney test.

3. Results

3.1. Bacteria identified pre- and post-infestation with *P. ovis*

Before infestation with *P. ovis*, DNase-negative staphylococci were cultured from the skin of all seven sheep sampled and *Bacillus* spp. were cultured from four animals. *S. aureus* was not cultured from any of the seven sheep. Six weeks after infestation *S. aureus* was the predominant bacteria cultured from lesions on five of the ten sheep sampled. These five sheep were designated Group A. In three of these sheep a pure growth of *S. aureus* was obtained, in one a DNase-negative staphylococcus and in another a *Bacillus* spp. were also identified. The other five sheep were *S. aureus*-negative and were designated Group B. Pure cultures of DNase-negative staphylococci were obtained from lesions on these animals.

3.2. Bacterial counts pre- and post-infestation with *P. ovis*

Aerobic culture of samples obtained before and 6 weeks after infestation gave geometric means for the number of viable CFU of 8228 (95% CI, 832–165,959) and 647,590 (95% CI, 2630–15,135,612), respectively. Anaerobic culture of these samples gave geometric means for viable CFUs of 3376 (95% CI, 47–123,027) and 973,487 (95% CI of 4266–18,197,009), respectively, for colonies grown before and 6 weeks after infestation. Although there was a considerable difference between the geometric means of numbers of aerobic CFU before and after infestation and anaerobic CFU before and after infestation, the difference was not statistically significant.

3.3. Lesional area and clinical signs

Six weeks after infestation the geometric mean for the lesional area in *S. aureus*-positive sheep (Group A) was 541 cm² (95% CI, 219–1130 cm²) and 421 cm² (95% CI, 168–836 cm²) in *S. aureus*-negative sheep

(Group B). There was no statistical difference in lesional areas of Groups A and B sheep.

On examination of cutaneous lesions 6 weeks after infestation, abundant yellow crusts were observed at the advancing edge of the lesion and the underlying skin was inflamed and covered by exudate. In addition, the lesions were intensely pruritic. However, clinically no difference could be detected in lesions of Groups A and B sheep.

3.4. SDS-PAGE and Western immunoblotting

3.4.1. *S. aureus* extract

Blots of *S. aureus* extract were probed with sera collected, before and 6 weeks after infestation, from five *S. aureus*-positive (Group A) and five *S. aureus*-negative sheep (Group B) and the binding of IgG and IgE isotype antibodies in these sera to reduced *S. aureus* antigens and allergens, respectively, was investigated.

In Group A pre-infestation sera from two sheep demonstrated IgG immunoreactivity against an 65 kDa band. Six weeks after infestation sera from all Group A sheep demonstrated IgG immunoreactivity to bands of 36, 38, 50 and 65 kDa (Fig. 1) although in one animal the immunoreactivity was less intense. In contrast, pre- and post-infestation sera from four Group B sheep failed to demonstrate IgG immunoreactivity against *S. aureus* antigens. Pre- and post-infestation sera from one animal demonstrated IgG immunoreactivity against bands of 40, 50 and 65 kDa (Fig. 2). Controls demonstrated non-specific binding of horseradish peroxidase to *S. aureus* antigens of 80 and 130 kDa.

No IgE immunoreactive bands were detected with pre- or post-infestation sera from any of the sheep in Groups A and B.

3.4.2. *P. ovis* WME

Blots of *P. ovis* WME were probed with sera collected, before and 6 weeks after infestation, from five *S. aureus*-positive (Group A) and five *S. aureus*-negative sheep (Group B) and the binding of IgG and IgE isotype antibodies in these sera to reduced *P. ovis* antigens and allergens, respectively, was investigated.

In Group A, pre-infestation sera from two sheep demonstrated IgG immunoreactivity against a 220 kDa band and another animal possessed weak

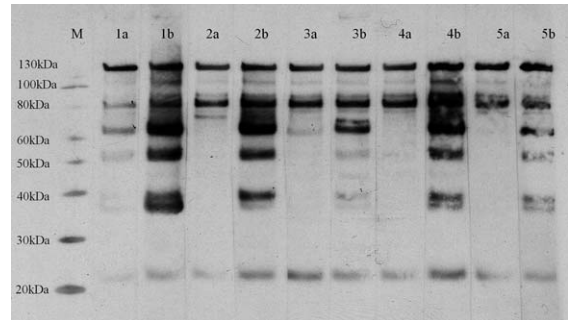


Fig. 1. Blots of *S. aureus* extract probed with sera from Group A sheep (1–5). Pre-infestation sera from two sheep (1a and 3a) demonstrated IgG immunoreactivity against a 65 kDa band. Six weeks after infestation sera from all sheep demonstrated IgG immunoreactivity to bands of 36, 38, 50 and 65 kDa. Controls demonstrated non-specific binding of horseradish peroxidase to *S. aureus* antigens of 22, 80, 100 and 130 kDa. M, molecular weight marker; 1a to 5a are pre-infestation sera; 1b to 5b are post-infestation sera.

IgG immunoreactivity to a 100 kDa band. Six weeks after infestation sera from all Group A sheep demonstrated marked IgG immunoreactivity against bands of 10, 30, 90 and 220 kDa. In addition, sera from four sheep also bound to antigens of 40 kDa and sera from three sheep reacted with antigens of 28 kDa (Fig. 3). In Group B, pre-infestation sera from three sheep demonstrated distinct IgG immunoreactivity against 100 kDa band and serum from one of these animals also displayed IgG immunoreactivity against

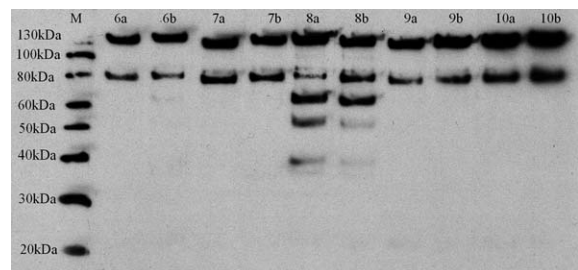


Fig. 2. Blots of *S. aureus* extract probed with sera from Group B sheep (6–10). Pre- and post-infestation sera from four Group B sheep (6, 7, 9 and 10) failed to demonstrate IgG immunoreactivity against *S. aureus* antigens. Pre- and post-infestation sera from sheep 8 demonstrated IgG immunoreactivity against bands of 40, 50 and 65 kDa. Controls demonstrated non-specific binding of horseradish peroxidase to *S. aureus* antigens of 80 and 130 kDa. M, molecular weight marker; 6a to 10a are pre-infestation sera; 6b to 10b are post-infestation sera.

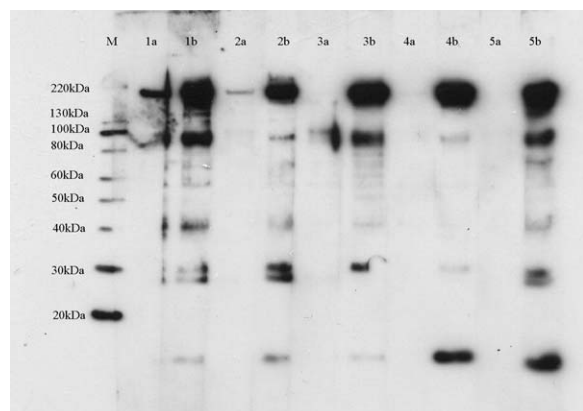


Fig. 3. Blots of *P. ovis* WME probed with sera from Group A (1–5). Pre-infestation sera from two sheep (1a, 2a) demonstrated IgG immunoreactivity against a 220 kDa band and another animal (3a) possessed weak IgG immunoreactivity to a 100 kDa band. Post-infestation sera all Group A sheep demonstrated marked IgG immunoreactivity against bands of 10, 30, 90 and 220 kDa, sera from four sheep (1b, 2b, 3b and 5b) also bound to antigens of 40 kDa and sera from three sheep (1b, 2b and 5b) reacted with antigens of 28 kDa.

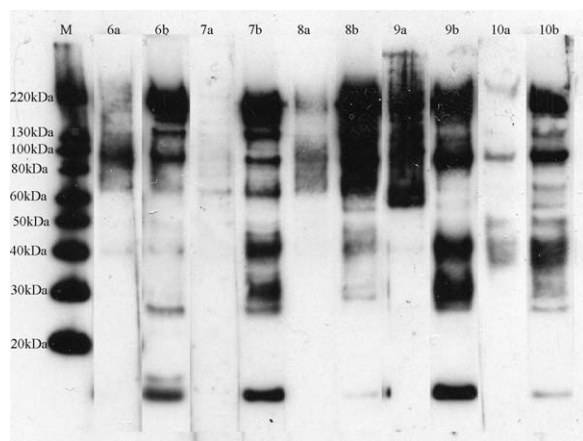


Fig. 4. Blots of *P. ovis* WME probed with sera from Group B (6–10). Pre-infestation sera from three sheep (6a, 8a and 10a) demonstrated distinct IgG immunoreactivity against 100 kDa band and serum from one of these animals (8a) also displayed IgG immunoreactivity against a 220 kDa. Post-infestation sera of all sheep demonstrated IgG immunoreactivity against bands of 10, 90 and 220 kDa but a definitive 30 kDa band was detected by sera from only two sheep (7b and 9b). In addition, sera from three sheep reacted with 40 kDa (7b, 8b and 10b) and 28 kDa (6b, 7b and 10b) antigens and serum from two sheep reacted with antigens of 60 kDa (7b and 10b).

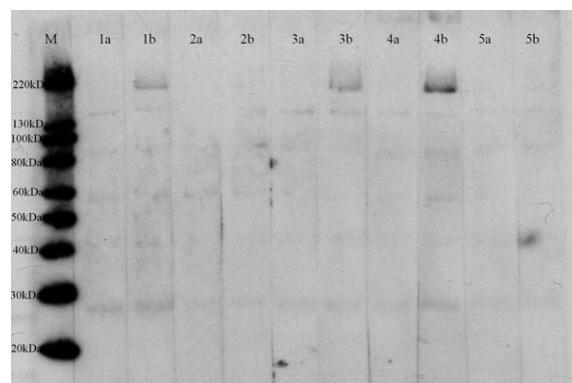


Fig. 5. Blots of *P. ovis* WME were probed with sera from Group A before and 6 weeks after infestation. Pre-infestation sera (1a to 5a) demonstrated no IgE immunoreactivity. However, 6 weeks after infestation weak IgE immunoreactivity with a 180 kDa band was detected in sera of three sheep (1b, 3b and 4b). M, molecular weight marker; 1a to 5a are pre-infestation sera; 1b to 5b are post-infestation sera.

a 220 kDa. Six weeks post-infestation all sheep demonstrated IgG immunoreactivity against bands of 10, 90 and 220 kDa but a definitive 30 kDa band was detected by sera from only two sheep. In addition, sera from three sheep reacted with 40 and 28 kDa antigens and serum from two sheep reacted with antigens of 60 kDa (Fig. 4).

Pre-infestation sera from Group A sheep demonstrated no IgE immunoreactivity. However, 6 weeks after infestation weak IgE immunoreactivity with a 180 kDa band (Fig. 5) was detected in sera of three Group A sheep. No IgE immunoreactivity was detected in pre or post-infestation sera from animals in Group B.

4. Discussion and conclusion

An earlier study indicated that vaccination of sheep with live and killed *S. aureus* provoked an IgG isotype antibody response but did not define the IgG-reactive antigens (Kerlin and Watson, 1987). A major observation made in the present study was that secondary infection of sheep scab lesions by *S. aureus* elicited a specific IgG response against *S. aureus* proteins with molecular weights of approximately 36, 38, 50 and 65 kDa. The failure of IgG in sera from four of the five Group B (*S. aureus*-negative lesions with a

heavy growth of DNase-negative staphylococci) to recognise these proteins suggests that they are *S. aureus*-specific antigens. In addition, the absence of IgG-reactive bands of similar molecular weight in blots of *P. ovis* proteins indicates that cross-reactivity between *S. aureus* and *P. ovis* antigens is unlikely. The detection of IgG immunoreactivity against *S. aureus* proteins in pre-infestation sera from two Group A and one Group B animal may indicate a previous *S. aureus* infection. However, as 29% of healthy dairy sheep carried *S. aureus* in the nares (Vautor et al., 2005) it is possible that some clinically normal sheep mount an IgG response to the bacteria. Certainly this would be consistent with the observation that in humans healthy individuals carrying *S. aureus* in the nares may have an IgG response comparable to that seen in patients with staphylococcal disease (Bell et al., 1987). In the present study no evidence of *S. aureus*-specific IgE was detected. Significantly higher mean levels of *S. aureus*-specific IgE have been detected in cases of human atopic dermatitis with secondary colonization by *S. aureus* (Leung, 1997; Yamada et al., 1996) but *S. aureus*-specific IgE has not been detected in patients with psoriasis and chronic *S. aureus* colonization (Leung, 1997). It is, therefore, possible that production of IgE-inducing exotoxins is determined by environmental conditions (Schlievert and Blomster, 1983) and that conditions in the sheep scab lesions were not suitable. It is also possible that the limited period of our study was insufficient to allow the production of detectable amounts of *S. aureus*-specific IgE although Western blots did provide evidence of *P. ovis*-specific IgE at 6 weeks after infestation.

The influence of *S. aureus* on the pathogenesis of sheep scab lesions remains to be elucidated. However, in the present study, IgE reactivity with a 180 kDa *P. ovis* allergen was detected 6 weeks after infestation only in sera from *S. aureus*-positive sheep. This 180 kDa band corresponds to apolipoprotein/ vitellogenin one of three recently characterised *P. ovis*-allergens (Huntley et al., 2004) and its detection 6 weeks after infestation with *P. ovis* is consistent with previous observations (Huntley et al., 2004; van den Broek et al., 2003). Detection of an IgE response to this allergen only in *S. aureus*-positive sheep may indicate that *S. aureus* enhances the IgE response against *P. ovis* and would be consistent with the

observation that *S. aureus* augments allergen-specific IgE in atopic patients (Hofer et al., 1999) and mice (Matsui and Nishikawa, 2003). The role of *S. aureus* in promoting an IgE response to *P. ovis* allergens and the development of resistance of sheep to *P. ovis* (van den Broek et al., 2000) merits further investigation as studies in cattle have suggested that induction of IgE-mediated Type I responses appears to improve resistance to experimental infestations with *P. ovis* (Pruett et al., 1998). The Western blots of *P. ovis* proteins probed to detect IgG reactivity yielded results comparable to those obtained in previous studies (van den Broek and Huntley, 2003) and confirmed the diversity of individual responses to *P. ovis* antigens (van den Broek and Huntley, 2003). *S. aureus* has been cultured from *P. ovis* mites harvested from *S. aureus*-positive lesions and infected mites may therefore provide a source of *S. aureus* antigens that contaminate Western blots of *P. ovis*. However, in the present study, as no bands with molecular weights that corresponded to those of the *S. aureus* proteins were detected on blots of *P. ovis* antigens probed with sera from *S. aureus*-positive sheep, contamination by bacteria in the mites clearly was not significant.

In this study the predominant bacteria cultured from clinically normal skin of sheep before infestation with *P. ovis* were DNase-negative *Staphylococcus* spp. and *Bacillus* spp. This is consistent with earlier reports of the occurrence of *Staphylococcus* spp. including, *S. epidermidis* (Bates, 2003; Chin and Watts, 1992), *S. xylosus* (Lyness et al., 1994) and *S. cohnii* (Lyness et al., 1994) which rarely produces DNase and *Bacillus* spp. such as *B. pumilus* (Bates, 2003), *B. thuringiensis* (Lyness et al., 1994) and *B. cereus* and *B. coagulans* (Chin and Watts, 1992) as part of the normal sheep skin bacterial flora. *S. aureus* has also been cultured from the clinically normal skin/fleece of sheep (Chin and Watts, 1992; Meyer et al., 2001) although it was not isolated in other studies (Bates, 2003; Lyness et al., 1994) or the present one. However, in the present study, in half of the animals *S. aureus* was a predominant isolate from lesional skin after infestation with *P. ovis*. This bacterium has been associated previously with sheep scab lesions (Billingsley, 2003), including staphylococcal furunculosis (Yeruham et al., 2002). *S. aureus* has also been reported to be aetiological agent of staphylococcal

dermatitis in sheep (Fraser et al., 1982; Jansen and Hayes, 1987; Scott et al., 1980; Synge et al., 1985) and it has been demonstrated experimentally that intra-dermal inoculation of *S. aureus* caused suppurative lesions which resembled those observed in field cases (Fraser et al., 1982). The source of the *S. aureus* in these cases is probably the nares as it has been isolated from this site in up to 29% of healthy dairy sheep (Vautour et al., 2005). Healthy humans may also carry *S. aureus* in anterior nares (Kluytmans et al., 1997). Our observations suggest that the nares and possibly other sites act as reservoirs for *S. aureus* from which it is distributed to the skin where it becomes established when local conditions such as the inflammation and self trauma associated with *P. ovis* infestation provide a favourable environment.

In the present study, the major clinical signs were pronounced erythema, exudation, exuberant crust formation, alopecia and pruritus. These clinical signs are similar to those reported earlier in sheep infested with *P. ovis* (Sargison, 1995; Scott, 1988; van den Broek and Huntley, 2003). Although it has been suggested that the presence of bacteria exploiting the local pathological environment may exacerbate and influence the development of scab lesions (Hamilton et al., 2003), in the present study no clinical differences were observed in the clinical appearance or area of *S. aureus*-positive and -negative lesions. This is consistent with observations made of lesions in human patients with atopic dermatitis which although frequently colonized by considerable numbers of *S. aureus* (Hauser et al., 1985; Matsui and Nishikawa, 2003) manifest no significant clinical evidence of this. Although the present study failed to demonstrate any difference in clinical signs or lesional areas in *S. aureus*-positive and -negative sheep, *S. aureus* clearly provoked a significant immune response and may also have influenced the nature of the inflammatory infiltrate. Further histochemical and immunohistochemical studies are required to investigate this.

It is evident that *S. aureus* frequently colonizes lesions caused by *P. ovis* infestation and that this colonization elicits a specific IgG isotype antibody response and may also promote the induction of IgE responses to *P. ovis* allergens. Further studies are necessary to characterise the IgG-reactive *S. aureus* proteins and to investigate the role of *S. aureus* in the immunopathogenesis of sheep scab lesions.

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