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Research paper

# Cutaneous cytokine gene expression and cellular responses in lambs infested with the louse, *Bovicola ovis*, and following intradermal injection of crude louse antigen

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ABSTRACT

The present study was undertaken to investigate further the immunological responses in the skin of lambs to natural louse infestation and following intradermal allergen challenge. *Bovicola ovis*-infested ($n = 7$) and naïve ($n = 7$) Romney lambs received four intradermal injections each of crude louse Ag and diluent control solutions on the dorso-lateral chest. From each lamb, skin samples were obtained from untreated skin and, at 4, 24, 48 and 72 h following injection, from one each of the Ag- and diluent-injected skin sites. Levels of acetylcholinesterase-positive Langerhans and MHC II$^+$ cells in the epidermis as well as MHC II$^+$, CD1b$^+$, T19$^+$ and IgE$^+$ cells, eosinophils, and diffuse IgE staining in the dermis were significantly elevated in infested compared to naïve lambs (all $p \leq 0.01$). Additionally, gene expression of interleukin-4 (IL-4), IL-5, IL-13 (all $p \leq 0.001$) and IL-10 ($p \leq 0.05$) was significantly higher in the skin of infested compared to naïve lambs while TNF-α and IFN-γ gene expression were not significantly different between the two groups. Intradermal injection of louse Ag led to immediate and late phase responses in the infested lambs while the naïve lambs showed only minimal responses. Levels of dermal MHC II$^+$, CD1b$^+$, T19$^+$and IgE$^+$ cells, eosinophils and diffuse IgE staining in infested lambs following injection of louse Ag were similar to or exceeded those in untreated skin and, with few exceptions, were higher than in naïve lambs. Additionally, cytokine gene expression of IL-4, IL-5, IL-13 and IL-10 increased to peak levels 4 h following Ag injection in the infested lambs ($p \leq 0.001$, $\leq 0.05$, $\leq 0.05$ and $\leq 0.001$ respectively compared to untreated controls) and remained significantly elevated compared to that observed in the naïve controls for the duration of the experiment. Significant elevations of MHC II$^+$ cells and IL-4, IL-5, IL-13 and IL-10 gene expression were observed in the louse-naïve lambs following injection of louse Ag but were much less pronounced than in the infested lambs. These results indicated that louse infestation in lambs elicited a highly skewed Th2 immuno-inflammatory response with many characteristics similar to those seen with other parasitic infections and also in atopic dermatitis.



## 1. Introduction

Infestation of sheep with the louse, *Bovicola ovis*, is common in sheep-producing countries throughout the world. The economic impacts of louse infestation include reduced quantity and quality of wool harvested and the costs of measures taken to control infestation (McLeod,

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Abbreviations: Ace$^+$, acetylcholinesterase-positive; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HE, haematoxylin and eosin; PBSG, PBS plus 1.6% glycerol; Ct, cycle threshold value.

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1995). Infestation with the louse also leads to a skin disease, known in lay terms as scatter cockle, which devalues pelts especially of young sheep (Halligan and Johnstone, 1992; Heath et al., 1995). Scatter cockle is most obvious on processed pelts where it is characterised by numerous discoloured raised lumps, up to 1 cm in diameter, with a distribution corresponding to the predilection sites of the louse. The lesions *in vivo* present as papules and often have a punctuate depression or small crust in the centre (Pfeffer et al., 1996a).

The immunological and morphological features of natural cockle lesions and the responses to challenge with *B. ovis* Ag in lambs, in general, are characteristic of responses to external and internal parasites (Heath et al., 1995; Balic et al., 2000; van den Broek and Huntley, 2003a; Milnes et al., 2007; Pfeffer et al., 2007). However, *B. ovis* is regarded as a surface feeder, browsing on debris on the skin and wool fibres (Sinclair et al., 1989). It appears likely that allergens from the louse, particularly those shed in the faeces, elicit an immunological response following penetration of the skin barrier. In this regard, cockle may be analogous to atopic dermatitis in which lesions are elicited by the penetration of allergen from the skin surface (Maeda et al., 1992; Kapp, 1995; Olivry et al., 1997). It is not surprising then that natural cockle lesions and responses of infested lambs to *B. ovis* Ag challenge also show many similarities to atopic dermatitis (Pfeffer et al., 2007).

Microscopically, hypertrophy and hyperplasia of the epidermis and perivascular to diffuse leukocyte infiltration is observed in the superficial dermis of lambs with cockle (Halligan and Johnstone, 1992; Heath et al., 1995; Pfeffer et al., 1996a). Eosinophils are numerous in the infiltrate and are mixed with mononuclear leukocytes including lymphocytes, plasma cells, and macrophages.

Louse-infested lambs show immediate and late phase skin responses following intradermal challenge with crude *B. ovis* Ag and basophils from infested lambs show specific histamine release *in vitro* when challenged with this Ag (Pfeffer et al., 1994, 1997, 2007). Specific proliferative responses of lymphocytes from the skin-draining, prescapular lymph nodes of infested lambs were also induced by *B. ovis* Ag *in vitro* but little or no response was observed with lymphocytes from peripheral blood or lymph nodes not draining skin (Bany et al., 1995a,b). Changes in morphology and key cytokine gene expression in skin following intradermal challenge with louse Ag showed many similarities to those observed following intradermal challenge with allergens in human and canine species with atopic disease (Pfeffer et al., 2007). In the latter species, cytokine gene and protein expression in skin from atopic dermatitis lesions and late phase responses following allergen challenge were typically of the Th2 pattern initially while IFN-γ expression may be up-regulated in the chronic stages (Grewe et al., 1998; Sinke et al., 2002; Nuttall et al., 2002). However, in skin of louse-infested lambs following *B. ovis* Ag challenge, IL-4 gene expression was increased but no change in IFN-γ gene expression was observed (Pfeffer et al., 2007).

The present study confirms and extends our previous results on cytokine gene expression by examining interleukin-4 (IL-4), IL-5, IL-10, IL-13, TNF-α and IFN-γ in biopsy samples of untreated skin and following intradermal challenge with *B. ovis* Ag in further groups of louse-infested and naïve lambs. The levels of acetylcholinesterase-positive ($Ace^+$) Langerhans cells; MHC $II^+$, $CD1b^+$, $T19^+$ and $IgE^+$ leukocyte subsets; eosinophils, and diffuse IgE staining in the skin samples are also described.

## 2. Materials and methods

### 2.1. Animals and experimental design

Romney lambs, approximately 12 months old, and either naturally louse-infested ($n = 7$) or louse-naïve ($n = 7$) were derived from flocks of louse-infested or louse-free ewes respectively for the present experiment as previously described (Pfeffer et al., 1997). Intradermal skin testing was undertaken as previously described (Pfeffer et al., 2007). Each lamb received four intradermal injections of crude *B. ovis* Ag (each 100 μl, 100 μg/ml protein in PBS plus 1.6% glycerol (PBSG)) on the lateral thorax in a line parallel to the long axis of the lamb. In a line below the Ag injections, four intradermal injections of PBSG (diluent control, 100 μl) and one of histamine diphosphate, (control for histamine responsiveness, 100 μl, 10 μg/ml in PBS, Sigma Chemical Co., St. Louis, MO) were given at the same time. As responses to Ag and PBSG were consistent across sites in individual lambs, the most caudal Ag and PBSG sites and the histamine site on each lamb were measured at 30 min, 4, 24, 48 and 72 h after injection to provide data on the development of the skin reactions. The magnitude of the skin responses at each time was determined by measuring the longest diameter and the diameter perpendicular to this using a digital calliper ('Digimatic', Mitutoyo, Japan). At the same time a score from 0 to 4 was given to each skin response based on estimates of thickness; 0 = no swelling; 1 = 1–2 mm; 2 = 3–4 mm; 3 = 5–6 mm and score 4 ≥ 7 mm. The representative volume of each skin response at each time was expressed as units, calculated by the product of the two diameters (mm) and the thickness score +1. The louse score (number of live lice seen in twelve 10 cm partings of the fleece) of each lamb was determined at the beginning of the experiment and cockle was scored on the shorn skin at the sites prepared for intradermal injections (scale of 0–4 based on extent and density of lesions) as previously described (Pfeffer et al., 1997). The use of lambs in these experiments was approved by the AgResearch Wallaceville Animal Ethics Committee.

### 2.2. Skin biopsy

Skin biopsy samples were taken from one Ag and one PBSG injection site, choosing the most cranial unsampled site each time, at each of 4, 24, 48 and 72 h following injection as previously described (Pfeffer et al., 2007). One sample of untreated shorn skin was taken from each lamb as a non-treatment control. In the lousy lambs, the latter samples were taken from macroscopically obvious lesions of cockle (Pfeffer et al., 1996a) in 6 of the 7 lambs.

One half of each biopsy sample was snap frozen in liquid nitrogen for histological studies. The other half of