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Identification of auto-antigens in skin scrapings from scabies-infected pigs

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Abstract

Sarcoptes scabiei continues to cause major health and economic problems in a large range of animals and humans. Although the inflammatory response to the mite and its antigens is known to cause the main pathology, little work has been carried out on this response at the site of infection. This report presents an initial analysis of the proteins found in skin scrapings and their antigenic responsiveness in pigs. Skin scrapings and mite extracts were isolated from chronically infected sows while infected and uninfected sera were isolated from pigs with confirmed infections or mange-free pigs, respectively. Electrophoresis and sequencing confirmed the main components of both the skin and mite extracts to be serum proteins. Immunoblotting then suggested that transferrin was the major antigen recognised by pooled infected sera in the skin and the mite extracts. Immunoassays confirmed that a majority of infected pigs produced antibodies to transferrin while mange-free pigs did not. A pool of IgG from infected dogs was then used to isolate another antigen from pig skin scrapings which was shown to be haptoglobin. This was also found to induce high titres of antibody in infected pigs as compared with mange-free pigs. The use of albumin as a control antigen showed no reactivity in either group of sera. The finding of two iron-binding molecules as strong auto-antigens in pig scabies has implications for the importance of iron during this infection and may help to explain the persistence and magnitude of the host inflammatory response.

Keywords: Scabies; Pigs; *Sarcoptes scabiei*; Auto-antigens; Iron binding; Transferrin; Haptoglobin

1. Introduction

Scabies is a highly contagious skin disease of humans and other mammals caused by the parasitic mite *Sarcoptes scabiei*. Periodic outbreaks of scabietic mange occur in a wide variety of domestic and wild animal species and can cause morbidity and mortality in many of these species (Arlian, 1989; Bornstein et al., 2001).

Scabies is characterised by intense skin inflammatory reactions and these are the major cause of economic losses and health effects (Davies, 1995; Kemp et al., 2001). These reactions are caused by mite products that include a number of potent immunogens, some of which are shared with dust mite species such as Dermatophagoides sp. (Arlian et al., 1991; Fischer et al., 2003). These antigens have been characterised by a number of authors and are now being cloned and analysed via genetic and proteomic techniques (Ljunggren et al., 2003; Fischer et al., 2003). There has been less attention to antigens at the site of the inflammatory reaction in the skin except that antibody responses have been characterised (Arlian et al., 1996) as has the presence of Complement C3, IgG, IgA, IgM and IgE in the local vasculature, in the epidermis and the dermoepidermal junction (Frentz et al., 1977; Hoefling and Schroeter, 1980; Salo et al., 1982). However, these responses would seem to be only part of the complex inflammatory reactions described as occurring during mite infections (Arlian, 1989).

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Skin scrapings have not been generally examined as a source of scabies antigens or of host inflammatory components, probably due to the difficulties in separating mite from host material. However, skin scrapings offer a number of benefits for the analysis of host responses and for antigen identification in that they contain the various host reactive components including antibodies, acute phase proteins and other components of the host/mite interaction. In addition, any detectable antigens are ipso facto in contact with the host immune system and thus their impact on the host should be measurable.

Analysis of the host components and antigens released in skin will give a more detailed knowledge of the symptomatic inflammation and pruritis that accompanies scabies, may allow the development of antigen-based diagnostic assays and may lead to novel control methods. This paper reports an analysis of skin scrapings from infected pigs, the identification of their major components including two auto-antigens and the prevalence of antibodies against these auto-antigens in pigs.

2. Materials and methods

2.1. Preparation of soluble protein porcine skin scraping extract

All work with animals was approved through the relevant animal ethics procedures at both La Trobe University and the Pig and Poultry Research Institute. Skin scrapings were obtained from mange-infected pigs at piggeries (Lameroo, South Australia, Australia and Corryong, Victoria, Australia) and a local pig abattoir (Hurstbridge, Victoria, Australia). Pigs suffering chronic mange were identified and the inner ear scraped to dislodge thick scabs, loosely attached to the skin, as these scabs are known to be rich in mites (Cargill et al., 1997). The crusts were scraped from the ears, placed in a 50 ml plastic tube and kept on ice until processed. In the laboratory, 20 ml of cold PBS (16 mM Na_2HPO_4, 4 mM $NaH_2PO_4{\cdot}2H_2O$, 120 mM NaCl, pH 7.4) was added to the skin scrapings. The tube was vortexed until a homogenous solution of skin scrapings and buffer was achieved, then placed on ice for 6 h with periodic vortexing every 40 min. The homogenate was centrifuged for 10 min at 30,000*g*, aliquoted into 1 ml volumes and stored at −20 °C. The concentration of protein in soluble protein extracts was determined using a bicinchoninic acid (BCA) protein estimation kit (Pierce Chemical Company, Rockford, Illinois) according to the manufacturer's instructions.

2.2. Preparation of soluble protein Sarcoptes scabiei extract

Sarcoptes scabiei var. *suis* were isolated from skin scrapings using a modified method of Sheahan and Hatch (1975). Skin scrapings were cut into 1 cm^2 pieces and placed in a petri dish (Disposable Products, Adelaide, Australia). This was placed on a large flat aluminium tray, which was placed on the hotplate of a magnetic stirrer (Industrial Equipment and Control, Australia). The stirrer was set to the maximum setting to vibrate the tray, while the heat was set to the lowest setting to keep the tray warm. The temperature was monitored and kept at approximately 30 °C to prevent thermal damage to the mites and reduce the amount of condensation in the petri dishes. The tray was subjected to vibration and heat for 10 h to lure mites out of their burrows. A lamp was used to attract the mites to the side of the petri dish closest to the light. The crusts and most of the epidermal debris and hair were separated from the mites by inverting the dish, as the mites remained adherent to the dish by their ambulacral suckers. Mites were washed off the petri dishes using a 20 ml volume of cold PBS and then placed in a 50 ml plastic tube. The mites were washed three times in PBS with centrifugation at 1000*g* for 5 min. The same volume of PBS was then added and the tube was vortexed until a homogenous solution of mites and buffer was achieved. This was placed on ice for 6 h with periodic vortexing every 40 min. The homogenate was centrifuged for 10 min at 30,000*g* and then aliquoted into 1 ml volumes and stored at −20 °C. The concentration of protein in soluble protein extracts was determined using the BCA protein estimation kit.

2.3. Source and collection of pig sera

Serological testing was carried out on sera from pigs maintained at the Pig and Poultry Production Institute, South Australian Research and Development Institute, University of Adelaide, Roseworthy, South Australia. Mange-negative sera refers to pig sera taken from animals belonging to a herd monitored free of mange since 1970. Mange-positive sera refers to 122 days old pigs having natural infestations of *S. scabiei* var. *suis*. These pigs were confirmed to be infested with *Sarcoptes* through skin scrapings and other clinical markers for mange in pigs, such as ear lesion score and rubbing index. Blood was taken from the jugular vein of pigs and allowed to clot at room temperature for 2 h prior to centrifugation at 3000*g* for 15 min at 4 °C. The serum was collected and stored in aliquots at −20 °C.

2.4. Chromatography using mange positive dog IgG coupled to CNBr Sepharose

Dog sera from mange-infected dogs was kindly supplied by Catherine Wilkson (Murdoch University, Perth, Western Australia). Sera from seven chronically infected individuals were pooled and the IgG fraction purified from serum using Protein A Sepharose according to manufacturer's instructions (Amersham Pharmacia Biotech). CNBr-activated Sepharose 4B (Amersham Pharmacia Biotech) was prepared and used according to manufacturer's instructions. Purified mange-infected dog IgG (5 mg) was prepared in coupling buffer (0.1 M $NaHCO_3$, 0.5 M NaCl, pH 8.3) and added to the gel. The gel was incubated