FOLIA PARASITOLOGICA 41: 223–227, 1994

Serum antibody profiles of *Sarcoptes scabiei* infested or immunized rabbits

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Key words: *Sarcoptes scabiei*, immunization, antibody profile

Abstract. The circulating antibody profiles of rabbits infested or immunized with *Sarcoptes scabiei* var. *canis* were compared. Crossed immuno-electrophoretic analysis showed that infested hosts produced serum antibody to 12 proteins (antigens) in an extract made from sarcoptic mite bodies. In contrast, rabbits immunized with an extract made from mite bodies produced antibody to 20 *Sarcoptes* proteins (antigens). SDS-PAGE/immunoblot analysis revealed that serum from immunized rabbits contained antibodies that bound strongly to proteins of 25 and 39–52 kD that were only barely visualized by antibodies in serum from infested rabbits.

Laboratory and natural hosts infested with the ectoparasite *Sarcoptes scabiei* (SS) produce serum antibody against mite antigens (Arlian et al. 1985, 1994a, Arlian and Morgan 1994, Burgess 1994). Crossed immunoelectrophoresis (CIE) has been used to demonstrate that laboratory rabbits infested with *Sarcoptes scabiei* var. *canis* produce antibodies to at least 9 *Sarcoptes*-specific antigens (Arlian et al. 1985) while infested dogs and pigs show a similar response (unpublished). These mites burrow in the *stratum corneum* and are not usually in contact with living tissue. However, the burrowing mites produce and secret saliva (Arlian et al. 1984) and they deposit fecal and excretory matter into the burrow behind the anal opening. Other body secretions may also be produced. These expelled products contain antigenic molecules that reach the appropriate effector components of the immune system and this results in production of antibody by plasma cells. The observation of cellular infiltrate that was concentrated around the mouth parts and anal opening suggests that saliva and excretion/fecal material may be the primary source of the antigens and that these antigens induce an inflammatory/cell mediated response in the scabietic lesion and antibody production (Arlian et al. 1994b, c). Both laboratory and natural hosts express immunity to *Sarcoptes* infestation following a prior infestation (Mellanby 1944, Arlian et al. 1994a). Apparently, sensitization to some relevant antigens induces immune memory. It may be possible to both increase the efficacy of this immunity within a host and to increase the percentage of individuals in a population that express immunity by vaccinating with whole mite body extracts which would contain a larger antigen pool and thus expose the host to a greater spectrum of relevant antigens. For example, relevant antigens associated with other body processes (e.g., gut or hemolymph proteins) might be more efficient than naturally secreted antigens (saliva, feces, etc.) and these could be isolated and used in a vaccine. It seems likely that an infested host is exposed to very little, if any, antigen from inside the mite body, yet it may be these antigens which might be most useful in eliciting a protective response.

The purpose of this study was to compare the serum antibody response of hosts infested with *Sarcoptes scabiei* var. *canis* with that of hosts immunized with an extract produced from whole *Sarcoptes* mite bodies.

MATERIALS AND METHODS

Antigen extracts. An extract of *Sarcoptes scabiei* var. *canis* (SS) was prepared using mites collected from crusts harvested from the ears of previously infested rabbits (Arlian et al. 1985, 1988, 1991). Mites were ground and defatted in anhydrous diethyl ether. A weighed amount of mites was placed in a TenBroeck homogenizer along with 20 volumes of cold 20 mM Triton-X100. A homogenate was prepared by grinding with 20 strokes of the pestle and the solution was allowed to extract at 4°C for 48 hrs. Insoluble material was pelleted by centrifugation and the supernatant (extract) was collected. The pellet was subjected to a second extraction with 20 volumes of Triton and the supernatant was again collected. The extracts were pooled and sterile filtered (0.2 μm) into sterile vials. Protein content was determined using the Bradford assay with bovine serum albumin as the standard (Bradford 1976).

A second 1:20 (w/v) SS extract was prepared as above except that glass distilled water, rather than Triton, was used as the extractant.

Immunization protocol. On day 0, two mature New Zealand White rabbits were immunized with an emulsion of 1.0 ml of Triton-extracted SS (containing 2.7 mg protein) and

1.0 ml of Freund's Complete Adjuvant. The dose was evenly distributed among occipital, axillary and inguinal areas on each rabbit. At days 13 and 32, rabbits were boosted with the same doses as before except that emulsions were prepared with Freund's Incomplete Adjuvant (FIA). At days 60 and 74, rabbits were boosted with 1.0 ml of SS/FIA emulsion while 0.5 ml doses were administered at days 97 and 122.

Serum. Blood was collected from the central ear arteries of alternating ears of immunized rabbits at various intervals and the serum was processed as previously described (Arlian et al. 1985, 1988, 1991). Serum was stored at –20°C for later antibody analyses. Serum from each individual collection was evaluated using crossed immunoelectrophoresis (CIE) and ten samples with the highest antibody titres were pooled.

Serum from rabbits infested with *Sarcoptes scabiei* var. *canis* was as previously described (Arlian et al. 1985). A positive antiserum pool was made using serum samples from 36 individual infested rabbits. Each sample included in the pool recognized 5–10 antigens by CIE.

Naive (normal, control) rabbit serum was purchased from the Sigma Chemical Co. (St. Louis, MO, USA).

Crossed immunoelectrophoresis (CIE). The number of *Sarcoptes* antigens recognized by the two serum pools was evaluated using CIE as previously described (Arlian et al. 1985, 1988, 1991, 1994a, Arlian and Morgan 1994). Fifteen microlitres of sarcoptic mite extract (25 µg protein) was electrophoresed in the first dimension 1% agarose gel for 30 min at 200 V. Antigen-containing lanes were excised and placed transversely on a sheet of GelBond®. An upper gel (anodic) containing 300 µl of rabbit serum in 3 ml of 1% agarose and a lower gel (cathodic) with 60 µl of serum in 1 ml of agarose were poured and electrophoresed overnight at 50 V. Gel plates were washed, pressed, dried and stained with Coomassie Brilliant Blue R-250.

SDS-PAGE/immunoblotting. The number of *Sarcoptes* antigens recognized by the two serum pools was also studied using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblotting. Electrophoresis was carried out under non-reducing (no reducing agent added to sample buffer) and reducing (2-mercaptoethanol added) conditions using the method of Laemmli (1970). Kaleidoscope® Prestained Standards (Bio-Rad, Richmond, CA, USA) were used to provide molecular weight markers. Prior to transfer, a strip from each gel was removed for subsequent protein detection by silver staining (Arlian et al. 1985, 1988, 1991).

PAGE-resolved proteins were electrophoretically transferred to Pro-Blott® PVDF membrane (Applied Biosystems, Foster City, CA, USA) in 10 mM CAPS, 10% methanol, pH 11 according to the manufacturer's recommendations. Membranes were rinsed in glass-distilled water and a strip with lanes of both *Sarcoptes* proteins and molecular weight markers was excised from each (non-reduced and reduced) and stained with 0.1% Amido Black. The remainder of each membrane was cut into 1 cm strips each containing the equivalent of 20 µg of mite-derived protein. Strips were blocked with 5% nonfat dry milk in PBS with 0.5% Tween 20 (PMT) (Arlian and Morgan 1994, Arlian et al. 1994a). Strips were incubated in varying dilutions of *Sarcoptes*-infested, *Sarcoptes*-immunized or normal rabbit serum in PMT for 2 hours at room temperature. Serum specific antibody binding was detected by peroxidase-labeled goat anti-rabbit Ig (Fisher Scientific) at 1:2,000 in PMT. All strips were developed for 4 min. using the CND (4-chloronapthol/3,3'-diaminobenzidine/H_2O_2) chromogenic substrate of Young (1989).

RESULTS

Crossed immunoelectrophoretic (CIE) analysis revealed that rabbits chronically infested with *Sarcoptes scabiei* var. *canis* synthesized antibodies to 12 antigens. The typical CIE profile of *Sarcoptes* extract reacted with serum from infested rabbits showed 5 distinct, darkly-staining, anodally-migrating antigen-antibody precipitin peaks and an approximately equal number of faint, weakly-staining peaks (Fig. 1A, C). The pattern was the same for water or Triton-prepared mite extracts.

In contrast, CIE analysis of serum obtained from rabbits immunized with extracts of *Sarcoptes scabiei* var. *canis* showed a much more complex antibody profile. The reaction of a sarcoptic mite extract, prepared with or without Triton, with the antiserum from *Sarcoptes*-immunized rabbits resulted in 20 precipitin peaks. Ten peaks exhibited intense Coomassie blue staining

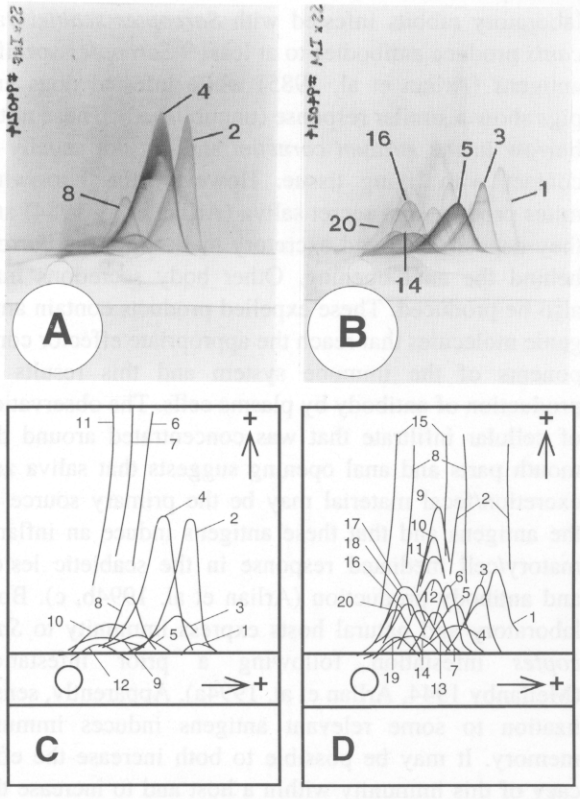

Fig. 1. Homologous CIE gels of an extract of *Sarcoptes scabiei* var. *canis* reacted with serum from *S. scabiei*-infested (A, C) or *S. scabiei*-immunized rabbits (B, D). Figures C and D are schematic representations of the Coomassie blue stained gels shown in figures A and B, respectively.

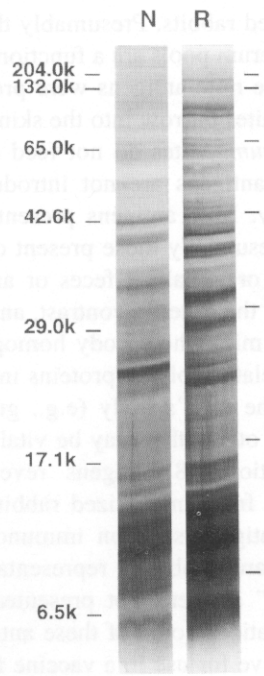

Fig. 2. Silver stained, 12% SDS-PAGE gels of *Sarcoptes* extract run under non-reducing (N) and reducing (R) conditions. Locations of molecular weight markers are shown for reference.

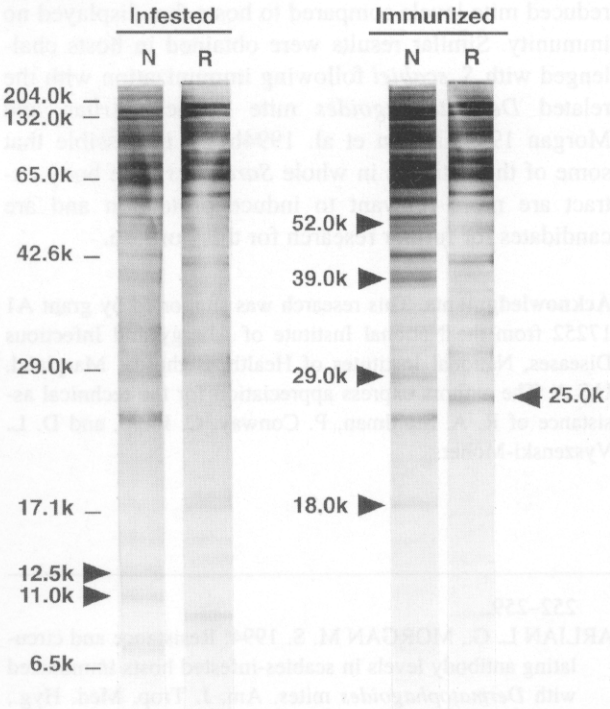

Fig. 3. Immunoblots of *Sarcoptes* extract resolved on 12% SDS-PAGE gels under non-reducing (N) and reducing (R) conditions. Blots were probed with serum from *S. scabiei*-infested or *S. scabiei*-immunized rabbits at a dilution of 1:2,000. Locations of molecular weight markers are shown for reference and molecular weights of bands of interest are indicated with arrows.

while a similar number showed much fainter staining (Fig. 1B, D). Reaction of *Sarcoptes* extract with naive (never infested or immunized) rabbit serum did not result in the formation of any immunoprecipitates.

To further characterize the antibody profiles of the two serum pools, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblotting was employed. Total protein staining of the gel strips showed >50 protein/peptide bands under both non-reducing and reducing conditions (Fig. 2). Immunoblots probed with serum from both *Sarcoptes*-infested and immunized rabbits showed binding to >25 bands under both sets of conditions (Fig. 3). The patterns were very similar with several notable exceptions. Serum from infested rabbits showed binding to bands of 12.5 and 11.0 kD under non-reducing conditions that were not observed when immunized serum was used to probe the strips. Conversely, bands of 18.0 and 29.0 kD were seen on the non-reduced strip probed with immunized serum while a pronounced band at 25.0 kD was observed on the blot under reducing conditions. Additionally, serum from *Sarcoptes*-immunized rabbits showed pronounced binding to a series of bands from 39–52 kD for which only faintly-stained matching bands were visualized with serum from infested rabbits. Blots probed with serum from naive (never infested or immunized) rabbits showed no binding to sarcoptic mite antigens.

DISCUSSION

On the basis of CIE analysis of individual serum samples from 5 rabbits chronically infested with *Sarcoptes scabiei* var. *canis*, we previously concluded that these hosts produced circulating antibodies to at least 9 antigens present in an extract prepared from the bodies of *Sarcoptes* mites (Arlian et al. 1985). In the present study, using CIE to study antibodies in a serum pool prepared using individual serum samples from 36 infested rabbits, we identified 12 antigens to which chronically infested rabbits produced antibodies. Presumably burrowing mites in the *stratum corneum* secreted/excreted sufficient amounts of these antigens to stimulate the hosts to synthesize antibody.

In contrast to the antibody response of infested rabbits, CIE analysis suggested that rabbits immunized with an extract of sarcoptic mite bodies produced antibodies to at least 20 individual antigens present in this extract. This is not surprising, since similar extracts of the phylogenetically related house dust mites *Dermatophagoides farinae*, *D. pteronyssinus* and *Euroglyphus maynei* all elicit the production of >30 antibodies when used to immunize rabbits (Arlian et al. 1987a, b, 1988, 1991, 1993). The significant degree of immunologic

cross-reactivity between *Sarcoptes* mites and the *Dermatophagoides* mites (Arlian et al. 1988, 1991) would suggest that *Sarcoptes* mite extracts would also contain many antigenic proteins. Other studies have shown that extracts made from bodies of unfed ticks contain antigens not present in extracts of salivary glands (Brown and Askenase 1986, Wikel and Whelen 1986, Willadsen et al. 1988). In ticks, a membrane-bound glycoprotein gut antigen in extracts of homogenized tick bodies has been shown to induce protective immunity in vaccinated hosts (Willadsen et al. 1989).

SDS-PAGE/immunoblot analysis of these two serum pools confirmed that there were distinct differences in the antibodies present. Several proteins/peptides were recognized as antigens by serum from immunized but not from infested rabbits. Serum from *Sarcoptes*-immunized rabbits also contained antibodies which exhibited pronounced binding to a group of bands from 39–52 kD which were only barely detected by antibodies in serum from infested rabbits. At identical dilutions, many bands on the strips probed with serum from immunized rabbits were darker than corresponding bands on strips probed with serum from infested rabbits. This suggested that serum from immunized rabbits contained higher titres of antibodies directed at these antigens.

The discrepancy in the number of antigens visualized by CIE and SDS-PAGE was likely due to differences in the two analytical techniques employed. The detergent treatment, heat and reducing agent used in the SDS-PAGE method of Laemmli (1970) denatures proteins and reduces them to their component peptides. Proteins composed of multiple antigenic peptides are therefore detected as multiple antigen bands on immunoblot analysis. In CIE, however, proteins are not denatured so that CIE is a reflection of the number of intact antigenic molecules (not fragments) recognized by the serum sample.

Regardless of the analytical method used, serum from *Sarcoptes*-immunized rabbits clearly recognized more mite proteins as antigens than did serum from sarcoptic mite infested rabbits. Presumably the differences in these two antiserum pools are a function of the differences in which the mite antigens were presented to the rabbits. Scabies mites burrow into the skin and reside in the *stratum corneum*. Mites do not feed on blood and hence *Sarcoptes* antigens are not introduced into the host intravenously. Mite antigens presented to the infested host are presumably those present on the surface of the mite body or in saliva, feces or any other substance secreted by the mite. In contrast, an extract made from a *Sarcoptes* mite whole body homogenate should contain all mite-related soluble proteins including those normally inside the mite's body (e.g., gut and hemolymph proteins or others that may be vital for mite survival). The additional 8 antigens revealed in CIE analysis of serum from immunized rabbits and the 25 and 39–52 kD antigens seen on immunoblots probed with this serum are probably representatives of this group of "hidden" antigens not presented to the host during mite infestation. Some of these antigens may be particularly effective for use in a vaccine for protection against scabies. We have previously shown that infestation with *S. scabiei* does induce moderate levels of immune-based protection during subsequent reinfestation (Arlian et al. 1994a, c). Sixty-five percent of challenged hosts exhibited resistance as evidenced by much reduced mite levels compared to hosts that displayed no immunity. Similar results were obtained in hosts challenged with *S. scabiei* following immunization with the related *Dermatophagoides* mite extract (Arlian and Morgan 1994, Arlian et al. 1994b). It is possible that some of the antigens in whole *Sarcoptes* mite body extract are more relevant to induce protection and are candidates for further research for this purpose.

Acknowledgments. This research was supported by grant A1 17252 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, U.S.A. The authors express appreciation for the technical assistance of R. A. Stuhlman, P. Conway, C. Rapp, and D. L. Vyszenski-Moher.

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Received 3 May 1994

Accepted 1 July 1994