

## IDENTIFICATION OF A HOMOLOGUE OF A HOUSE DUST MITE ALLERGEN IN A cDNA LIBRARY FROM *SARCOPTES SCABIEI* VAR. *HOMINIS* AND EVALUATION OF ITS VACCINE POTENTIAL IN A RABBIT/*S. SCABIEI* VAR. *CANIS* MODEL

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**Abstract.** *Sarcoptes scabiei* (“itch mite”) causes scabies, a disease of considerable human and veterinary significance. Little work has been done at the molecular level because of the difficulty of obtaining mites. We have used mites in skin from the bedding of crusted scabies patients for the construction of a library of 10<sup>5</sup> cDNAs from *S. scabiei* var. *hominis* cloned in the vector pGEX4T-2. We describe the isolation by immunoscreening of 2 clones, one of which (Ssag1) is homologous to and cross-reactive with the house dust mite *Euroglyphus maynei* allergen M-177, an apolipoprotein from hemolymph. Immunohistochemistry revealed that it is located around the internal organs and cuticle of the mite and in eggs. Although it was not found to be protective in a challenge trial, the rabbits did not exhibit typical crust characteristics. This work shows that it is now possible to conduct such challenge trials with cloned scabies antigens.

### INTRODUCTION

Scabies (“itch mite,” or *Sarcoptes scabiei*) is a common infestation in the developing world,<sup>1–4</sup> with about 300 million people affected. Although it can be treated,<sup>5</sup> it is endemic among some disadvantaged populations, such as members of remote northern and central Australian Aboriginal communities,<sup>6</sup> in which 50% of children may be infested with *S. scabiei*. These infestations decrease the quality of life by causing intense itching and often lead to bacterial skin infections followed by serious complications, including septicemia and renal damage.<sup>7</sup> Secondary infections with Group A streptococci have been associated with outbreaks of rheumatic heart disease.<sup>8</sup> Scabies is also a problem in other crowded situations, such as nursing homes and day care centers. Historically, rapid increases in incidence have been noted during times of war and social upheaval. A vaccine is the most desirable long-term solution because of limitations of drug delivery and compliance.

In most cases of scabies, the mite burden is self-limiting in humans, suggesting that it is controlled immunologically. Mellanby<sup>9</sup> determined that in experimental infestations, the average number of mites on adults was 11 to 13. Reinfection was more difficult, and the parasite rate was even lower. A few people develop the serious alternative form, crusted scabies, characterized by an extremely high burden of mites. Outbreaks of scabies in hospitals after admission of such patients show that this is caused by the same variety of mite that causes normal scabies. Crusted scabies commonly occurs in patients with acquired immunodeficiency syndrome and other immunosuppressive conditions, but it also occurs in people with no known immunodeficiency.<sup>10</sup> It has been postulated that there is a specific immune deficit that results in a few individuals developing this hyperinfestation.<sup>11</sup> Scabies progresses to the crusted state in most animals if it is untreated, however.

Mites ingest rabbit antibodies during feeding; it has been shown by immunologic staining of sections that rabbit antibodies are present in the gut of the mite (LG Arlian et al., unpublished data). We have postulated that these antibodies in the mite gut may be protective.<sup>11</sup> Immunity after primary infestations has been shown in an animal model,<sup>12,13</sup>

and experimental immunization with mite extracts can induce protective immunity.<sup>14</sup> Taken together, these observations suggest that antibodies may play a role in the development of protective immunity. Mechanical removal of mites by scratching also can play an important role in control of mite numbers. The epidemiology of scabies in populations suggests partial immunity is acquired with age and exposure. It may be possible to control scabies in children using a vaccine.

Until more recently, there has been no literature on the molecular biology of scabies, owing to the difficulty of obtaining sufficient mites. A system of growing dog-derived mites on the ears of New Zealand white rabbits, which provides access to *S. scabiei* var. *canis* and allows the testing of potential vaccines, has been developed.<sup>14,15</sup> At the Royal Darwin Hospital, about 10 patients per year are admitted with crusted scabies. Shed skin from the bedding of these patients provides a noninvasive source of up to 4,000 mites per gram of skin, and we previously have cloned *EcoRI* genomic DNA fragments of *S. scabiei* var. *hominis* from this material.<sup>16</sup> Microsatellite clones from this library were used to show that *S. scabiei* var. *canis* and *S. scabiei* var. *hominis* are distinct populations regardless of their geographic origin.<sup>17</sup> Sequence analysis of the second internal transcribed spacer of the ribosomal RNA complex<sup>18</sup> nevertheless suggested that *Sarcoptes* mites are the same species. This suggestion was supported by sequencing a mitochondrial ribosomal RNA gene,<sup>19</sup> although both these studies were limited. A library of cDNA clones from *S. scabiei* var. *vulpes* has been constructed from mites obtained from red foxes, and a cDNA clone encoding paramyosin has been characterized.<sup>20</sup> The sequences of many other cDNAs from this library have been placed in GenBank (accession numbers BG817579–BG817974).

We report here the construction of cDNA libraries from *S. scabiei* var. *hominis*. We describe the isolation by immunoscreening of 2 clones, one of which is the *S. scabiei* homologue of a major house dust mite allergen. Although these clones showed no protective efficacy in the rabbit/*S. scabiei* var. *canis* model, the work shows that it is now possible to test cloned scabies antigens for vaccine potential. Future applications of these new resources are discussed.

## MATERIALS AND METHODS

**Harvesting scabies mites.** Ethics clearance for collection of skin samples containing mites and for collection of serum for the work described here was obtained from the Human Research Ethics Committee of the Menzies School of Health Research and the Royal Darwin Hospital, approval 97/21, 16/5/97. The use of human serum in the crossed radioimmuno-electrophoresis (CRIE) experiments was approved by the Human Subjects Committee of Wright State University (Assurance number M-1021; ID No. 01NR; Human Subjects Protocol No. 0205).

Live *S. scabiei* mites of various life stages were harvested from skin scrapings and bedding of crusted scabies patients admitted to the Royal Darwin Hospital before treatment. Samples were transferred to a glass petri dish, and mites were hand picked from the skin using a metal probe at a magnification of  $\times 40$  under a dissecting microscope.

**Isolation of mRNA.** Purification of mRNA was carried out using a QuickPrep Micro mRNA Purification Kit (Amersham Pharmacia Biotech, Castle Hill, NSW, Australia), according to the manufacturer's instructions. Initially, approximately 1,000 mites were homogenized in 50  $\mu$ l of extraction buffer using a microfuge tissue grinder and centrifuged briefly, and the supernatant was stored at  $-70^{\circ}\text{C}$ . After processing, the purified mRNA was ethanol-precipitated in the presence of glycogen carrier, resuspended in 30  $\mu$ l of diethyl pyrocarbonate-treated water and stored at  $-70^{\circ}\text{C}$ .

**cDNA synthesis and cloning into pGEX4T-2.** The method used to synthesize double-stranded oligo-dT primed and randomly primed cDNA from mite mRNA was a modification of the basic procedure described by Efstratiadis et al.<sup>21</sup> Double-stranded cDNA was prepared by priming with oligo-dT and by random priming.

Polyadenylated mRNA from about 100 mites was reverse transcribed independently from either an oligo-dT primer or random decamers and copied into double-stranded cDNA. After ligation of linkers PH019/020 (see later), the cDNA was amplified by polymerase chain reaction (PCR) using PH019 and oligo-dT ( $95^{\circ}\text{C}$  for 1 minute, then 30 cycles of  $95^{\circ}\text{C}$  for 30 seconds,  $45^{\circ}\text{C}$  for 30 seconds, and  $60^{\circ}\text{C}$  for 4 minutes). After PCR, the cDNA was digested with *EcoRI* to generate 5' sticky-ends. Double-stranded cDNA fragments  $>300$  bp were ligated into pGEX4T-2 (Amersham Pharmacia Biotech, Castle Hill, NSW, Australia), which had been digested with *EcoRI* and *SmaI*. After phenol/chloroform extraction and dialysis, recombinants were electroporated into *Epicurian Coli* Electroporation-Competent SURE Cells (Stratagene, La Jolla, CA) and selected on ampicillin-containing plates. Approximately  $10^5$  clones were obtained for each of the random primed and oligo-dT primed libraries.

**PH019/020 linker sequence.** Linker sequences were as follows: PH019—5' CGTGGATCCCCAGGAATTCCTGGG 3'; PH020—3' GCACCTAGGGGTCCTTAAGGGCCC 5'.

**Isolation of clones by antibody screening.** The method used to treat filters before colony immunoassays was as described by Laurenti et al.<sup>22</sup> Hyperimmune serum from rabbits infested with *S. scabiei* var. *canis* or immunized with scabies extract was prepared as described.<sup>23</sup> Specific binding was detected with antirabbit IgG (whole molecule) conjugated to alkaline phosphatase using NBT/BCIP chromogenic substrates (Promega Corporation, Madison, WI). Clones de-

tected in an initial screen of 90,000 clones were purified by 3 rounds of plating followed by colony assays, then used for affinity purification of the glutathione S-transferase-fused polypeptides over a Glutathione-Sepharose 4B column (Amersham Pharmacia Biotech, Castle Hill, NSW, Australia) according to the manufacturer's instructions.

**Immunologic methods.** Ethical approval for production of antibodies in rabbits was obtained from the Animal Ethics Committee of the Northern Territory University, Darwin, Australia (approval No. A99019). Approval for the vaccination/challenge trial and for the collection of serum from rabbits during the experiment was obtained from the Laboratory Animal Care and Use Committee of Wright State University (Assurance Number A3632-01; Animal Use Protocol No. 413). The maintenance and care of rabbits complied with the National Institutes of Health guidelines and the Australian National Health and Medical Research Council guidelines for the humane use of laboratory animals.

Rabbit antisera to the fused polypeptides were prepared at the Veterinary Services Division of the Institute of Medical and Veterinary Sciences, Gilles Plains, South Australia. The rabbit antisera to the fused polypeptides were characterized by immunoblotting, which was carried out as described.<sup>24,25</sup> Minor modifications include the overnight transfer of proteins onto a PVDF-Plus Transfer Membrane (Amersham Pharmacia Biotech, Castle Hill, NSW, Australia) in 0.1% SDS transfer buffer at 50 mA and  $4^{\circ}\text{C}$ .

Crossed immunoelectrophoresis (CIE) and CRIE were performed as previously described.<sup>23,26</sup> Briefly, 5  $\mu$ g of fusion protein was electrophoresed in the first dimension 1% agarose gel for 30 minutes at 200V. Antigen-containing lanes were excised and placed transversely on a sheet of GelBond. An upper gel (anodic) containing 150–400  $\mu$ l of rabbit serum in 3 ml of 1% agarose and a lower gel (cathodic) with 25  $\mu$ l of serum in 1 ml of agarose were poured and electrophoresed overnight at 50V. Gel plates were washed, pressed, dried, and stained with Coomassie Brilliant Blue R-250. To visualize IgE-binding allergens, unstained CIE gels were incubated overnight in serum from a crusted scabies patient diluted 1:250 followed by a second overnight incubation in 0.25  $\mu$ Ci of  $^{125}\text{I}$ -labeled antihuman IgE (Diamed, Windham, ME). Gels were washed and dried, and autoradiography was performed.

**Immunohistochemistry.** Tissue samples used were obtained from biopsy specimens from patients admitted to Royal Darwin Hospital for treatment of crusted scabies. Tissue sections containing scabies mites were adhered to silane slides after fixation in formaldehyde and embedding in paraffin. Mounted sections were dewaxed with 3 changes of histolene, then absolute alcohol, and endogenous peroxidase activity inactivated by incubation with 5%  $\text{H}_2\text{O}_2$  in methanol for 15 minutes at room temperature and in phosphate-buffered saline (PBS), pH 7.4 ( $2 \times 3$  minutes). To unmask tissue antigens that may have been cross-linked during fixation, sections were subjected to heat-induced microwave antigen retrieval by simmering in boiling Target Retrieval Buffer (citrate buffer, pH 6.0; DAKO Corporation, Copenhagen, Denmark) for 10 minutes in a microwave oven on low power. The sections were cooled to room temperature and rinsed twice in PBS as described. Skin sections were blocked with 3% normal goat serum in PBS for 15 minutes at room temperature. The blocking solution was drained, and primary rabbit antisera

(1:100–1:27,450 dilution in blocking solution) were applied to the sections for overnight incubation at room temperature in a humidified chamber. The sections were rinsed with PBS and overlaid with horseradish peroxidase (HRP)–conjugated biotinylated link antimouse and antirabbit immunoglobulin (for LSAB2 System, HRP; DAKO Corporation, Copenhagen, Denmark) for 10 minutes at room temperature, followed by streptavidin-HRP solution for a further 10 minutes. For staining of specific antibody binding sites in mite tissue, 3,3'-diaminobenzidine in chromogen solution was used. Sections were counterstained with Mayer's hematoxylin, differentiated in acid alcohol, then saturated lithium carbonate, dehydrated with absolute alcohol and histolene before clearing and mounting. Preparations were observed under a light microscope (Leica, Solms, Germany) at varying magnifications and photographed using Kodak 200 film (Coburg, VIC, Australia).

**Rabbit immunization and challenge trials.** A total of 500 µg of purified fusion protein was injected into each rabbit subcutaneously at several sites along the back and neck to minimize the reaction at each site and to ensure a good distribution of the material to all the lymph nodes of the rabbit. Up to 5 immunizations were performed, each 3 to 4 weeks apart. The primary dose was given as a 1:1 emulsion in Freund's complete adjuvant, and subsequent boosters were given as 1:1 emulsions in Freund's incomplete adjuvant. A blood sample was taken before the primary inoculation. The first test blood sample was taken 9 days after the third dose, and a final test sample taken after the last scheduled dose.

A total of 25 mature New Zealand white rabbits were immunized as follows: 5 controls (no immunogen), 5 adjuvant controls (water/Freund's), and 15 experimental (recombinant antigen/Freund's). At the completion of the immunization schedule, rabbits were allowed to rest for 2 months before being infested with *S. scabiei* var. *canis* mites on the medial surfaces of the left ear pinnae with crusts harboring approximately 2,400 live mites taken from previously infested animals. Blood samples were collected at 2, 4, 6, 8, and 9 weeks after infestation. The levels of scabies infestations were assessed by skin scrapings taken at 4, 8, and 9 weeks after infestation and counting the number of live mites present.

**Polymerase chain reactions.** PCRs were carried out as described<sup>27</sup> using the following primers: Ssag1, forward primer 5' CCGGGCCATCGAACGG 3' and reverse primer 5' CACTTCTCTTCGATTTACC 3'; Ssag2, forward primer 5' CCGGGGAAGAAGATAAGG 3' and reverse primer 5' TAGCGACTTTAATAATATCGG 3'.

**Sequencing.** pGEX4T-2 cDNA clones were cycle sequenced from the T3 and T7 primers by the dideoxy chain termination procedure using an AmpliCycle Sequencing Kit

(Perkin Elmer, Rowville, VIC, Australia) as per the manufacturer's instructions.

## RESULTS

**Initial characterization of the cDNA libraries.** The method for isolating mites from human skin crusts did not eliminate the possibility of contamination from human skin, and presumably there is human skin in the gut of the mites. The presence of human DNA clones in the library was anticipated but to what extent was not known. Clones from the oligo-dT and randomly primed pGEX4T-2 libraries were randomly selected and sequenced. Oligonucleotide primers specific for the inserts were designed and tested on DNA derived from mites and humans. Of the 6 clones initially tested, 3 amplified mite DNA only, 2 amplified products in mite and human DNA, and 1 amplified human DNA only (data not shown). The libraries predominantly contain scabies sequences with some human DNA contamination. This conclusion was confirmed by subsequent sequencing studies on a λZAP express library made from the same batch of mRNA (see the article by Fischer et al. in this issue).

**Isolation of Ssag1 and Ssag2 by antibody screening of the random primed library.** The first 2 clones isolated by immunoscreening the random-primed cDNA library with antisera from rabbits infested with var. *canis* mites were named *S. scabiei* antigen 1 (Ssag1) and Ssag2. After determination of the sequences, insert-specific forward primers were designed to check whether they were of mite origin. PCR with human DNA, mite DNA, and skin-plus-mite DNA extract with the Ssag1 and Ssag2 specific primers amplified products in mite DNA and skin-plus-mite DNA extract but not in human DNA, showing that both cDNAs were of mite origin (data not shown). The sequences were submitted to the GenBank expressed sequence tag (EST) database with the accession numbers BM176880 and BM176881.

The Ssag1 sequence was used to search the GenBank non-redundant database. A BLASTx search revealed a 70% amino acid sequence identity with Mag ( $E = 2 \times 10^{-44}$ ; accession No. P39673) and 67% identity with the *Euroglyphus maynei* M-177 high-molecular-weight allergen ( $E = 8 \times 10^{-42}$ ; accession No. AAF14270). An amino acid alignment of Ssag1 with the relevant region of the 1,668-amino acid M-177 sequence is shown in Figure 1. The Ssag2 sequence was almost identical to the sequence of an *S. scabiei* var. *vulpes* expressed sequence tag of unknown function recently entered into the database (BG817678), but had no other significant matches.

**Recombinant-Ssag1 and Ssag2 expression and properties.** To maximize expression levels of the recombinant antigens, the Ssag1 and Ssag2 plasmids were transfected into pro-

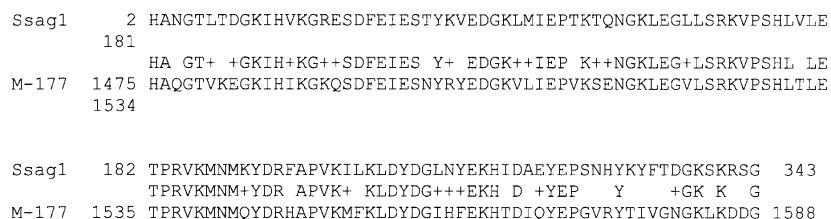


FIGURE 1. BLASTx alignment of the translated sequence of *S. scabiei* Ssag1 (BM176880, 344 bp) with the amino acid sequence of *E. maynei* M-177 (AAF14270, 1,668 amino acids). The identities are shown between the 2 sequences.

tease-deficient (*lon*<sup>-</sup>) *Escherichia coli* strain BL21 and expression levels compared with that in SURE cells. Ssag1 exhibited higher levels of expression in SURE cells, whereas Ssag2 was expressed to a greater degree in BL21 cells (data not shown). The fused polypeptides expressed in these cells from Ssag1 and Ssag2 were used for preparation of rabbit antisera, which were characterized by immunoblotting with the recombinant proteins. Antisera from these rabbits (1:1,000 dilution) showed strong specific binding to the corresponding purified fused polypeptide but bound minimally or not at all to the other fused polypeptide or to purified glutathione S-transferase from pGEX4T-2 (Figure 2). The antisera were suitable for immunohistochemical studies.

To confirm that Ssag1 was the target of an immune response in scabies-infested rabbits, the recombinant protein was incubated with serum from rabbits infested with *S. scabiei* var. *canis*, serum from rabbits immunized with a crude scabies extract,<sup>23</sup> serum from rabbits immunized with a crude scabies extract then infested with var. *canis*, or serum collected from rabbits at various times during the Ssag immunization/var. *canis*-challenge experiment. An antigen-antibody precipitate was observed by CIE using pooled serum from rabbits immunized with Ssag and rabbits immunized with a crude scabies extract (Figure 3A, B), and immunoblots were positive in each case. This finding is consistent with the identification of Ssag1 by screening with hyperimmune serum from infested rabbits. The precipitates bound IgE in serum from a scabietic human, showing that Ssag1 has an IgE-binding epitope (Figure 3C, D). Similar precipitates were observed with antisera from rabbits immunized with extracts from the house dust mites *Dermatophagoides farinae*, *D. pteronyssinus*, and *E. maynei* (data not shown), consistent with the identification of Ssag1 as the homologue of a house dust mite allergen.

**Immunohistochemical localization of Ssag1 and Ssag2.** Immunoperoxidase staining of sections through human skin, which was highly infested with *S. scabiei* mites, showed that anti-Ssag1 and anti-Ssag2 antibodies generated in rabbits bound strongly to the internal organs of the scabies mites and the cuticle. Minor staining of the surrounding skin was observed, but the signal generated from mites and eggs was much greater in intensity (Figure 4A–C). This result directly shows that the Ssag1 and Ssag2 antigens are mite components. The background with naive rabbit serum was quantitatively and qualitatively different from the antisera because it stained the mites and the human skin at much lower intensities (Figure 4D). Staining with different dilutions of the sera supported these conclusions (P Harumal, unpublished data).

**Immunization and challenge studies in a rabbit/*S. scabiei* var. *canis* system.** A mixture of both recombinant antigens Ssag1 and Ssag2 was used in the rabbit/*S. scabiei* var. *canis* model to determine whether a protective response was produced to fused proteins expressed from the cloned gene fragments. Immunoblot (data not shown) and CIE analysis (Figure 3) showed that rabbits produced antibodies against Ssag1 and Ssag2. On challenge infestations with var. *canis* mites, no reduction in the numbers of mites propagating on the rabbit ears was observed, however. The immunized rabbits did not develop a protective immune response to the cloned portions of Ssag1 and Ssag2. This conclusion suggests that in live mites, hemolymph proteins are not accessible to antibodies ingested into the gut or are not targets of protective immunity. The

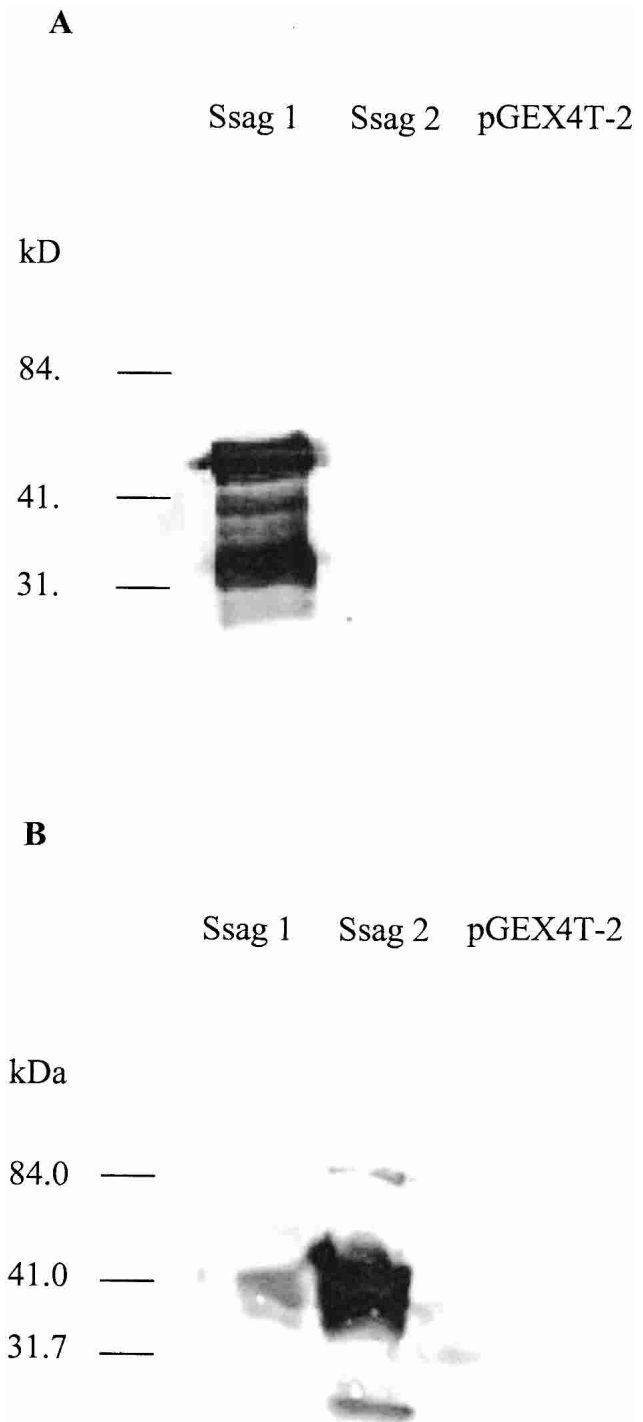


FIGURE 2. Immunoblotting fused polypeptides from Ssag1 and Ssag2 with antisera from rabbits immunized with (A) Ssag1 and (B) Ssag2.

immunized animals did not exhibit the typical crust characteristics compared with those exhibited by normally infested rabbits, however, perhaps a consequence of reaction of the antibodies with hemolymph proteins released from dead mites.

## DISCUSSION

We have overcome the limitations on availability of experimental material from which to generate *S. scabiei* var. *homi-*

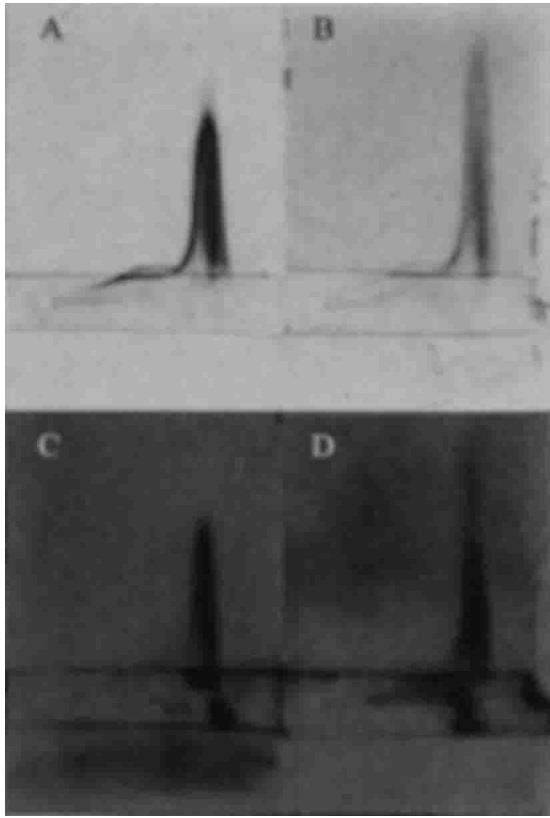


FIGURE 3. (A–D) Homologous and heterologous CIEs and CRIEs of Ssag1 protein. (A, C) Homologous gels precipitated using pooled serum from all Ssag immunized rabbits. (B, D) Heterologous gels precipitated using pooled serum from rabbits immunized with a crude scabies extract. (A, B) Coomassie blue-stained gels; (C, D) autoradiography films exposed for 2 days showing IgE binding by serum from a human crusted scabies subject.

*nis* cDNA libraries in part by using mites obtained from skin fragments in the bedding of crusted scabies patients. We constructed 2 cDNA libraries using PCR to amplify cDNA, which then was inserted into pGEX4T-2; we describe here the isolation and partial characterization of the first 2 clones identified by screening these libraries with antibodies from infested rabbits. With the fused polypeptides, we were able to prepare antibodies in rabbits. These antibodies allowed us to investigate some immunologic properties, to use immunohistochemistry to localize the gene products in the mite, and to investigate whether the recombinant antigens conferred protection against challenge.

Previous research on scabies at the molecular level has been constrained by the availability of experimental material. The availability of cDNA libraries from *S. scabiei* var. *vulpes*<sup>20</sup> and the libraries from *S. scabiei* var. *hominis* described here constitute a significant step forward. The scarcity of mite material and lack of an *in vitro* culture system have hindered further characterization of the Ssag1 and Ssag2 gene products, however, and so we have not been able to carry out successfully immunoblotting studies on extracts from *S. scabiei* var. *hominis*. There are further technical problems, such as high levels of human antibodies in protein extracts from hand-picked mites, presumably originating from the gut of the mites (unpublished data). Antigens involved in protective

immunity have not been identified, and it is not known whether there are sequence differences between those of *S. scabiei* var. *hominis* and *S. scabiei* var. *canis*, which would pose a problem in the challenge system.

The work described here has identified a homologue of a

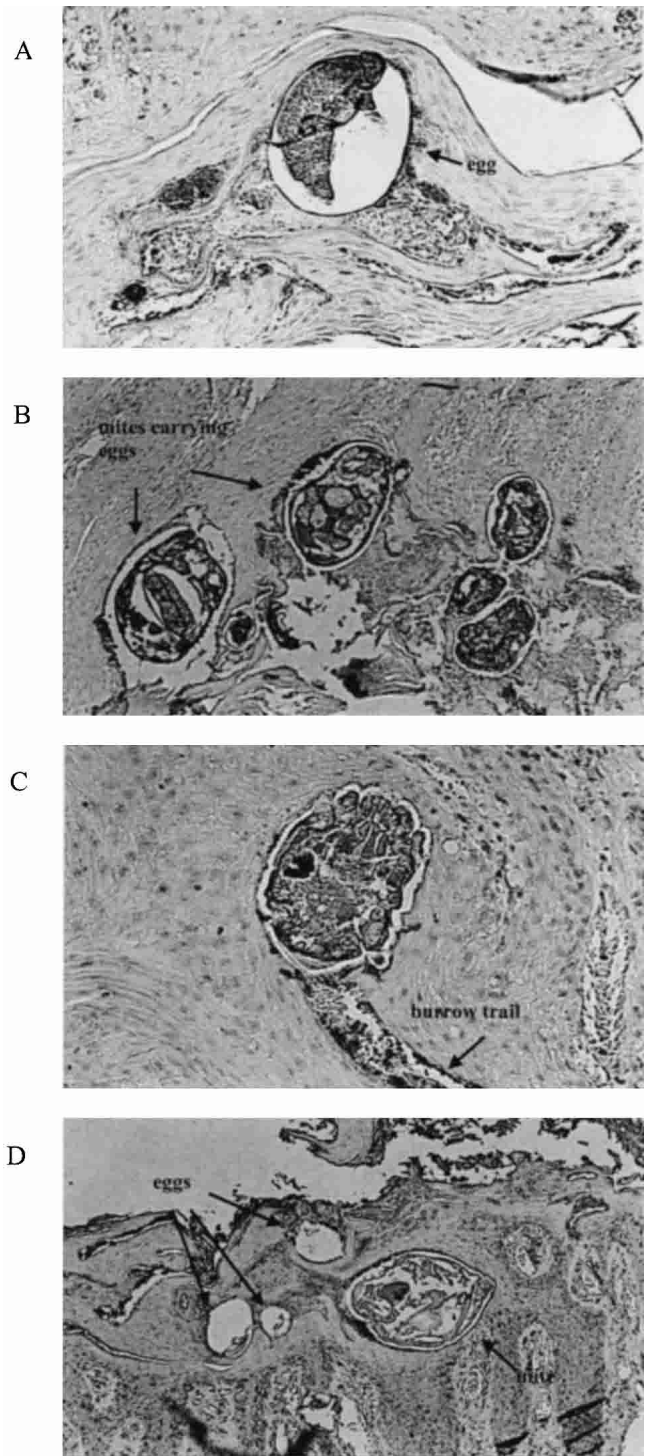


FIGURE 4. (A–D) Immunoperoxidase brown staining of skin sections after reaction with anti-Ssag1 sera. (A, C) Anti-Ssag1, 1:27,450 dilution (magnification,  $\times 250$ ). (B) Anti-Ssag1, 1:10,000 dilution (magnification,  $\times 100$ ). (D) Prebleed, 1:10,000 dilution (magnification,  $\times 100$ ).

major house dust mite allergen previously described in *D. farinae* as Mag<sup>28</sup> and Mag<sup>329</sup> and in *E. maynei* as the high-molecular-weight M-177.<sup>30</sup> This allergen is now known to be a hemolymph apolipoprotein.<sup>31</sup> Its homologue Ssag1, identified here by immunoscreening with serum from a rabbit infested with mites originating from dogs,<sup>23</sup> is a scabies mite gene product as shown by PCR amplification and by immunoperoxidase localization. It has potential as an immunodiagnostic molecule. This potential was confirmed by reaction of the purified fused polypeptide with sera from infested rabbits and rabbits immunized with extracts from mites. As would be expected from the high degree of sequence homology, Ssag1 cross-reacted with antisera prepared against 3 species of house dust mites. This result implies that there is >1 epitope on the Ssag1 portion of the fused polypeptide because formation of a precipitin band cannot occur without this. It appears to be an allergen in humans because it bears at least 1 IgE epitope, as shown by binding of human IgE to unreacted epitopes within the precipitin bands in Figure 3C and D. Studies to examine the sensitivity of a variety of constructs as immunodiagnostic molecules are warranted. The value in diagnosis depends, however, on the degree of cross-reaction with its homologue from house dust mites. The similarity in sequence observed here and the cross-reactivity with house dust mite antigens suggest that epitope scanning may be necessary to derive specific immunodiagnostic information.

As the sequences of many of the house dust mite allergens are available, the work described here suggests that searching for their homologues among a large number of scabies cDNA sequences would provide a rapid way of obtaining a library of such homologues. In the accompanying article by Fischer et al., we describe the construction and initial characterization of a *S. scabiei* var. *hominis* library cloned in the bacteriophage  $\lambda$  vector  $\lambda$ ZAP express for this purpose and the identification of 3 homologues of house dust mite allergens by random sequencing. The availability of libraries of cDNA clones, the beginning of an EST database derived from mites from humans and foxes, and the ability described here to carry out challenge trials with cloned antigens should facilitate greatly research on scabies.

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