

IDENTIFICATION AND CHARACTERIZATION OF *SARCOPTES SCABIEI* AND *DERMATOPHAGOIDES PTERONYSSINUS* GLUTATHIONE S-TRANSFERASES: IMPLICATION AS A POTENTIAL MAJOR ALLERGEN IN CRUSTED SCABIES

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Abstract. The astigmatid mite *Sarcoptes scabiei* is the causative agent of scabies, a highly infectious parasitic disease of the skin. Although the mite causes marked hypersensitivity reactions, particularly in crusted (severe) scabies, little is known about the specific scabies mite molecules involved in such immunologic responses. We have identified six genes encoding scabies mite homologues of mu and delta-like glutathione S-transferases (GSTs) as well as novel house dust mite GSTs. A mu class *S. scabiei* GST was subcloned into a prokaryotic expression system. The purified recombinant protein rSsGST01 reacted strongly with IgE and IgG4 in sera from crusted scabies patients. This response was not observed with control antigens or with ordinary scabies and uninfested patient sera. In addition, the specific IgE response to rSsGST01 did not correlate with the total IgE level of the patient. These results suggest that GST may play a role in the pathophysiology associated with crusted scabies.

INTRODUCTION

Scabies is caused by infestation of the skin with the “itch mite” *Sarcoptes scabiei*. Although the resulting pruritis causes significant distress, untreated scabies is also commonly associated with pyoderma, primarily caused by Group A *Streptococcus* and *Staphylococcus aureus* via damaged epidermis.^{1,2} The clinical consequences of secondary bacterial infection can include cellulitis, lymphangitis, and acute glomerulonephritis, resulting in considerable morbidity.² Further, there is increasing evidence that streptococcal infections of the skin can lead directly to rheumatic fever and consequent rheumatic heart disease.³ Scabies causes significant morbidity worldwide, especially for disadvantaged populations and immunocompromised individuals. It has been estimated that more than 300 million people are infested with *S. scabiei* despite the availability of effective treatments.⁴ In rare cases, crusted scabies develops, in which mites multiply in their millions and the affected person develops severe crusting of the skin. Like many other infectious diseases, scabies is transmitted by contact and therefore is primarily a disease of overcrowding and poverty. Scabies is endemic in northern and central Australian remote Aboriginal populations where up to 50% of children may be infested. Furthermore rates of crusted scabies in this population are among the highest recorded in the world.⁵

Little is known about immunologic responses in scabies. Pruritis and papular rash take 4 weeks or more to develop after primary infestation⁶ indicating a delayed type hypersensitivity response. However, lesions and itching are evident within 24–48 hours after secondary infestation. Both experimental infestation and clinical observations indicate resistance to infestation can be acquired.^{6–8} However, the individual antigens relevant to these responses have received very little study because of the extreme difficulty of obtaining experimental material in significant amounts. In the Northern

Territory of Australia, a limited number of patients (6–12 per year) with crusted scabies are admitted to the Royal Darwin Hospital. Shed skin from the bedding of these patients has provided a noninvasive source of mites, which has formed the basis of a DNA sequencing program. In recent immunoblotting studies, we demonstrated that sera from these patients showed strong IgE binding to up to 21 scabies mite proteins. In this study, it was found that sera from patients with ordinary scabies showed weaker binding to a maximum of six scabies mite proteins.⁹ However, the identity of these allergens is still unknown. This contrasts with the situation in the related astigmatid house dust mites, which can be cultured *in vitro*, and in which characterized antigens and allergens have been determined to be important in the development of asthma. Sequencing of cDNA clones prepared from scabies mites from foxes¹⁰ as well as those from the above patients with crusted scabies¹¹ have started to address this difficulty in the study of scabies. Scabies mite cDNA clones encoding homologues of a number of house dust mite allergens including the Group 11 paramyosin allergen,^{10,11} the Group 14 apolipoprotein allergen,^{11,12} the Group 8 glutathione S-transferase (GST) allergen,^{11,13} the Group 3 serine protease allergen,¹⁴ and the Group 1 cysteine protease allergen,¹⁵ have now been identified.

GSTs have been studied in a number of parasites as they are potentially relevant to both the immune response of the host and resistance of the parasite to drugs.^{16–18} They are ubiquitous in eukaryotes and catalyze the conjugation of glutathione to numerous electrophilic substances. They have a range of functions including metabolism of toxic substances, intracellular transport, digestive processes, and prostaglandin synthesis (reviewed in Refs. 19–21). The cytosolic GSTs are encoded by related gene families/classes including alpha, beta, delta, kappa, mu, omega, pi, sigma, theta, and zeta²² with interclass sequence identity generally lower than 30%.²³ Some classes have a wide distribution and have been identified in many organisms, whereas other classes appear to be more specific such as the beta class found in some bacteria, the phi and tau classes in plants, and the delta and epsilon

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classes in insects. At least nine classes of GSTs have been identified in mammals. Polyclonal antisera raised against a specific GST class will often cross-react with the same class from another species but cross-reactivity is generally not noted between classes.²⁴

Cytosolic GST from the house dust mite *Dermatophagoides pteronyssinus* (*Der p* 8, mu class)¹⁶ and the cockroach *Blattella germanica* (sigma class)²⁵ have been shown to act as allergens. IgE radioimmunoassays with the *Escherichia coli* lysate of a *D. pteronyssinus* cDNA clone showed that 40% of mite-allergic patients recognized the GST. More than half of that 40% show a reaction 10 times greater than that of the normal controls.²⁶ Recombinant *B. germanica* GST bound IgE in 70% of cockroach-allergic asthma patients.²⁵ Helminth GSTs have been reported to be effective vaccine antigens (reviewed in Refs. 27, 28). For example, *Schistosoma mansoni* GST induced IgE and IgA responses that were apparently effective in reducing parasitic load. The liver fluke *Fasciola hepatica* GSTs have been identified as novel vaccine candidates that protect sheep against fluke infection. A high percentage of *F. hepatica* GST peptides were found to be antigenic although considerable variability in response to the peptides was observed among the vaccinated sheep.²⁹

A mu class GST isolated from a salivary gland cDNA library from the cattle tick *Boophilus microplus* showed 40% identity to *Der p* 8.¹⁷ This GST was found to be expressed in salivary glands and the gut of partially and fully engorged females but not in larvae, from which a second *B. microplus* GST molecule was isolated.³⁰ A GST was also identified in the sheep scab mite *Psoroptes ovis* however neither IgE nor IgG antibodies could be detected to a recombinant form of the protein. In addition, intradermal injection of the recombinant protein did not induce a weal reaction or provoke the histopathological changes characteristic of the expected cutaneous inflammatory response.³¹

Because of the potential significance of mite GSTs in the host allergic immune response and their potential as effective vaccine antigens, we have examined the relationships between *S. scabiei* GST and *D. pteronyssinus* GST cDNA sequences identified in our EST databases. In this paper, we have conducted a phylogenetic analysis of these GST protein sequences and describe the expression of a mu class SsGST in *E. coli*. Additionally we present evidence of IgE and IgG4 immunoreactivity to this recombinant SsGST protein in patients with crusted scabies.

MATERIALS AND METHODS

Collection of patient sera. Collection of blood from patients was approved by the Human Research Ethics Committee of the Northern Territory Department of Health and Community Services and the Menzies School of Health Research (approval number 97/21). Informed consent was obtained and blood collected from four groups of adult patients: 1) active crusted scabies ("crusted," *N* = 10); 2) active ordinary scabies ("ordinary," *N* = 8); 3) previous ordinary scabies but currently uninfested ("uninfested," *N* = 10); and 4) no known exposure to scabies mites ("naïve," *N* = 10). The crusted scabies cases all had severe hyperkeratosis and numerous mites were seen on skin scrapings, whereas ordinary scabies patients had typical lesions and positive microscopic identification of a mite or mite body part. The clinical and

immunologic histories of the crusted scabies patients included in this study are described in Roberts and others.⁵ Briefly, all were from the tropical north of the Northern Territory of Australia and had been admitted to Royal Darwin Hospital for treatment of crusted scabies on multiple occasions. Total serum IgE levels of crusted scabies patients were obtained from Royal Darwin Hospital patient records.

Sequence identification and analysis. A total of 43,776 random sequences from *S. scabiei* cDNA libraries^{11,32} and 3,168 random sequences from *D. pteronyssinus* cDNA libraries¹⁵ were analyzed using the basic local alignment search tool (BLAST)³³ for homology to sequences in the GenBank non-redundant protein database, and assembled into contigs as previously described.¹³ Contigs with significant matches to GST molecules were translated and analyzed for known protein domains by searching against the NCBI conserved domain database. Amino acid sequence alignments were constructed using ClustalW,³⁴ a distance matrix produced using ProtDist and phylogenies estimated using the Kitch algorithm.³⁵ The contig chosen for expression, Yv4001A08 consists of 10 clones (with at least one from each of the three independent primary libraries) and the coding region has been sequenced with a minimum of fivefold redundancy.

Cloning, expression, and purification of recombinant GST in *E. coli*. The primers SsGST01-F 5'-gcgatccaaaACCG-TAAAAATGTCTTCG-3' and SsGST01-R 5'-ggcctgcagTTAATATTTTGTATTCC-3' (which included BamHI and PstI restriction sites, respectively; *S. scabiei* specific sequence shown in upper case) were used to amplify the full 689-bp coding sequence of the *S. scabiei* mu class GST Yv4001A08 (SsGST01) by polymerase chain reaction from the cDNA library using standard methods. The product was directionally cloned into the expression vector pQE-9 (QIAGEN, Clifton Hill, Victoria, Australia), transformed into the *E. coli* strain BL21 (containing pREP4) and clones sequenced in both directions and screened for the expression of an approximately 26-kDa protein. The recombinant SsGST01 protein was purified from the soluble fraction of the cell lysate by binding to a Ni-NTA agarose column (QIAGEN) and eluting under non-denaturing conditions (50 mM NaH₂PO₄, 300 mM NaCl, 250 mM imidazole, pH 8.0). As a control, recombinant *Schistosoma japonicum* 26-kDa GST (rSj26GST) from the plasmid vector pGEX4T-2 (Amersham Biosciences, Castle Hill, NSW, Australia) was expressed in *E. coli* strain BL21 and purified on a glutathione column according to the manufacturers instructions. Concentration of each of the proteins was determined using the Bradford Assay technique using Bradford reagent (Bio-Rad Laboratories, Reagents Park, NSW, Australia).

Generation of rabbit antisera against rSsGST01. Ethical approval for the use of rabbits for antibody generation was approved by the Animal Ethics Committee of the Northern Territory University, Darwin, NT, Australia (approval number A99019). Rabbit antisera was prepared at the Veterinary Services Division of the Institute of Medical and Veterinary Sciences, Gilles Plains, South Australia.

Gel electrophoresis analysis and immunoblotting. rSsGST01 and rSj26GST (2 µg/lane) were analyzed by sodium dodecyl-sulfate polyacrylamide gel electrophoresis using 13% reducing gels and staining with Coomassie Blue R250 (Sigma-Aldrich, Sydney, NSW, Australia), or by immunoblotting using standard methods.^{36,37} Primary rabbit antisera was diluted

1:500, and detected using 1:1,000 anti-rabbit immunoglobulins alkaline phosphatase conjugate (Sigma-Aldrich) and developed with BCIP/NBT substrate (Promega Australia, Annandale, NSW, Australia).

Enzyme-linked immunosorbent assays. An indirect enzyme-linked immunosorbent assay (ELISA) approach was used to detect specific antibody responses to the rSsGST01 and rSj26GST with human sera samples. Either 1 µg/well rSsGST01 or rSj26GST was bound to Nunc-Iummo 96-well plates with MaxiSorp surface (Nalge Nunc International, Rochester, NY) overnight at room temperature in phosphate-buffered saline (PBS). Plates were blocked with 0.5% bovine serum albumin in PBS for at least 2 hours at room temperature, then serum applied at a 1:10 (IgE/IgG4 assay) or 1:20 (IgG Assay) dilution in 0.5% bovine serum albumin in PBS and incubated overnight at room temperature. Between each step plates were washed once with 0.05% Tween 20 in PBS then twice with PBS. Secondary antibody conjugated to alkaline phosphatase, either 1:100 anti-human IgE (Sigma-Aldrich), 1:1,000 monoclonal anti-human IgG (Sigma-Aldrich), or 1:500 anti-human IgG4 (Southern Biotech, Birmingham, AL) diluted in 0.5% bovine serum albumin in PBS, was bound for at least 2 hours at room temperature. Color was developed using 4-nitrophenyl phosphate substrate (Roche, Dee Why, NSW, Australia) for 40 minutes at room temperature and the absorbance was taken as the geometric mean of triplicate absorbances measured at a wavelength of 405 nm.

To examine for any differences between group means results were statistically analyzed using non parametric Wilcoxon matched pairs and Mann-Whitney tests in GraphPad PRISM version 3.02.

To eliminate any competitive binding from IgG antibodies, IgE immunoassays were also performed with serum pre-absorbed against Protein G Sepharose 4 Fast Flow (Amersham Biosciences, Uppsala, Sweden). Briefly equal volumes of serum and prewashed protein G Sepharose, diluted 75:25 in PBS, were incubated on a roller at room temperature for 2 hours. The mixture was then centrifuged at $4,000 \times g$ for 1 minute, the serum removed, diluted 1/10 in blocking buffer, and IgE binding determined as previously described.

RESULTS

Identification of SsGST. Among the *S. scabiei* random cDNA sequences, 6 contigs consisting of a total of 64 clones were predicted by BLASTx and conserved domain searches to encode GST molecules. Two of the contigs, Yv4001A08 and Yv5004H11, had as their most significant BLASTx match the house dust mite *D. pteronyssinus* Group 8 allergen *Der p 8* (accession number AAB32224), a mu class GST. The contig Yv4001A08 included the sequence Yv4A08 (accession number AF462190), previously identified during a pilot study of 145 of the ESTs.¹¹ Yv4001A08 and Yv5004H11 were both predicted to encode 219 amino acid polypeptides, and appeared by all available criteria to be homologues of the mu class GST Group 8 allergens. Yv4001A08 and Yv5004H11 were 63% and 65% identical to *Der p 8*, respectively, and 77% identical to each other.

One further *S. scabiei* contig, Yv5001D03, had significant BLAST matches with mu class GSTs from various organisms, whereas the three remaining contigs, Yv4019D08,

Yv4022A06, and Yv5001F03, showed most significant matches to delta and theta class GST molecules from various organisms. Recent studies reveal Yv5001F03 is 100% identical to AY649788 an *S. scabiei* var. *vulpes* delta GST.¹³

Among the *D. pteronyssinus* random cDNA sequences, 3 contigs consisting of a total of 12 clones were predicted to encode GST molecules. The contig Dp7019C10 showed 97% amino acid identity to the published sequence of *Der p 8* and was presumed to be an allele of this molecule. Contigs Dp7002H05 and Dp7018E11 showed 73% and 26% amino acid identity to *Der p 8*, respectively, with the latter showing its most significant BLAST matches with delta, epsilon and theta class GSTs.

The sequences of the five *S. scabiei* and three *D. pteronyssinus* GST molecules were submitted to GenBank and were assigned the accession numbers AY825933–AY825940. The amino acid sequences of each of the contigs were aligned to each other and to mu, theta, delta and epsilon class GSTs from various organisms. The relationships between the sequences, estimated from the alignment, is shown in Figure 1. All of the *S. scabiei* and *D. pteronyssinus* sequences, which had as their most significant BLAST matches mu class GSTs, clustered with the known mu class sequences. Each possessed

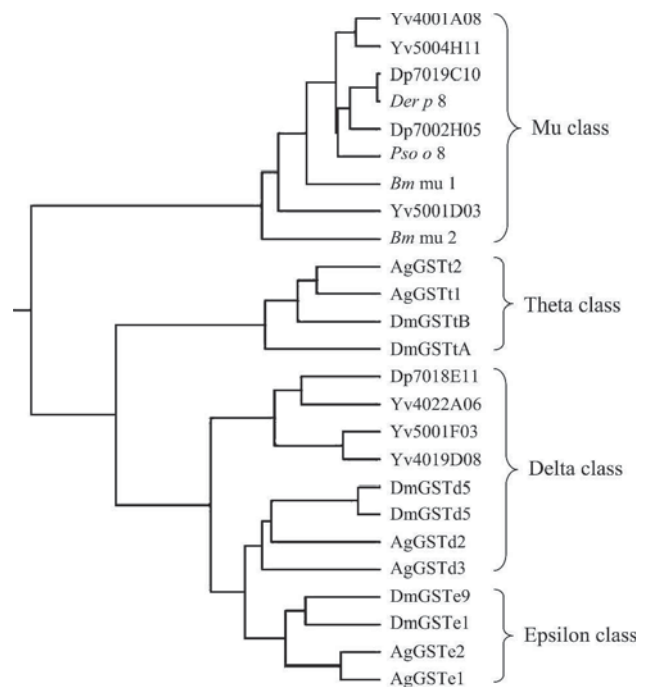


FIGURE 1. Relationships of the identified *S. scabiei* and *D. pteronyssinus* GST sequences with known GST sequences. The amino acid sequences of the identified molecules were aligned using ClustalW with mu class GSTs from *D. pteronyssinus* (*Der p 8*, P46419), *P. ovis* (*Pso o 8*, AAF19264), and *B. microplus* (*Bm mu 1*, AAD15991; *Bm mu 2*, AAL99403), as well as delta, epsilon, and theta class GSTs from *Drosophila melanogaster* (*DmGSTd2*, AAF54787; *DmGSTd5*, AAF54790; *DmGSTe1*, AAF57701; *DmGSTe9*, AAF57693; *DmGSTtA*, AAM50184; *DmGSTtB*, AAF50899) and *Anopheles gambiae* (*AgGSTd2*, CAA96104; *AgGSTd3*, AAM53610; *AgGSTe1*, AAG45163; *AgGSTe2*, AAG45164; *AgGSTt1*, AAM61893; *AgGSTt2*, AAM61892). Yv5001F03 is identical to recently released *S. scabiei* var. *vulpes* delta GST AY649788. A distance matrix was produced using ProtDist and phylogeny estimated using the Kitch algorithm.

a mu loop as well as the phenylalanine residue which acts as a "key" interacting with a "lock" provided by the second subunit in the dimer. Both of these are distinctive features of mu class GSTs.

All of the GST sequences identified possessed a residue in the N-terminal region proposed to be involved in catalysis. This consists of an invariant tyrosine in the mu class GST and a highly conserved serine in the delta and theta classes. Each also possessed the characteristic proline residue (in the loop between α -2 and -3), which, although not playing a direct role in catalysis, is thought to maintain the protein in a catalytically favorable conformation.³⁸

Expression and purification of recombinant *S. scabiei* GST in *E. coli*. The full coding sequence of the first identified *S. scabiei* GST contig Yv4001A08 (rSsGST01) was cloned into the vector pQE-9. rSsGST01 and rSj26GST were expressed in *E. coli* and purified under non-denaturing conditions by affinity chromatography. Analysis using polyacrylamide gel electrophoresis showed a single band of 26 kDa for the rSj26GST as expected and a single band of approximately 25 kDa for the rSsGST01 with no detectable contaminating proteins. Immunoblotting showed that polyclonal rabbit antisera raised against rSsGST01 recognised the rSsGST01 and also showed strong cross-reactivity with the rSj26GST (data not shown). The purified rSsGST01 bound to a glutathione column with no detectable level of unbound protein, and could be eluted from the column with glutathione, as could the purified rSj26GST. Thus presumably these recombinant molecules were in their native conformation and hence were suitable substrates for immunoglobulin binding studies.

IgE reactivity of human serum to rSsGST01 and rSj26GST. Serum from the four patient groups described were analyzed by ELISA to detect the presence of specific IgE immunoreactivity to rSsGST01 and rSj26GST (Figure 2). Using the

Mann-Whitney test to analyze for differences between group means, the IgE response of crusted scabies sera to rSsGST01 was significantly higher than the ordinary scabies sera ($P = 0.0085$), uninfested sera ($P = 0.0011$), and naive sera ($P = 0.0007$) (Figure 2). Furthermore, using the Wilcoxon matched pairs test, the IgE response of the crusted scabies group to rSsGST01 was significantly greater than their responses to rSj26GST ($P = 0.0059$). There were no significant differences between IgE binding to rSsGST01 and rSj26GST in the ordinary or naive sera groups; however, the uninfested sera demonstrated a significantly higher IgE response to the rSj26GST than to the rSsGST01 ($P = 0.0232$).

To determine if the IgE response to the rSsGST01 of each crusted scabies patient was due to a high total IgE level, the specific IgE response of each patient to rSsGST01 was compared with their total IgE level. Although the total level of IgE was extremely high in each serum, as has been previously shown in crusted scabies patients,³⁹ no correlation using non-parametric (Spearman) correlation analysis was found between the total IgE level of individual crusted scabies patients and the specific IgE response to rSsGST01 (Figure 3).

To evaluate whether SsGST01 specific IgE antibodies in ordinary and uninfested subjects were being masked/blocked by cross-reactive IgG, we further analyzed the same sera by ELISA after depletion of serum IgG with protein G immobilized on Sepharose beads. Depletion of IgG with protein G in crusted, ordinary, and uninfested patients did not increase specific IgE absorbance to rSsGST01 and rSj26GST antigen compared with when unabsorbed serum was used (data not shown).

IgG reactivity of human serum to rSsGST01 and rSj26GST. The crusted, ordinary and uninfested patient sera were also analyzed by ELISA for the presence of specific IgG immunoreactivity to rSsGST01 and rSj26GST (Figure 4). There

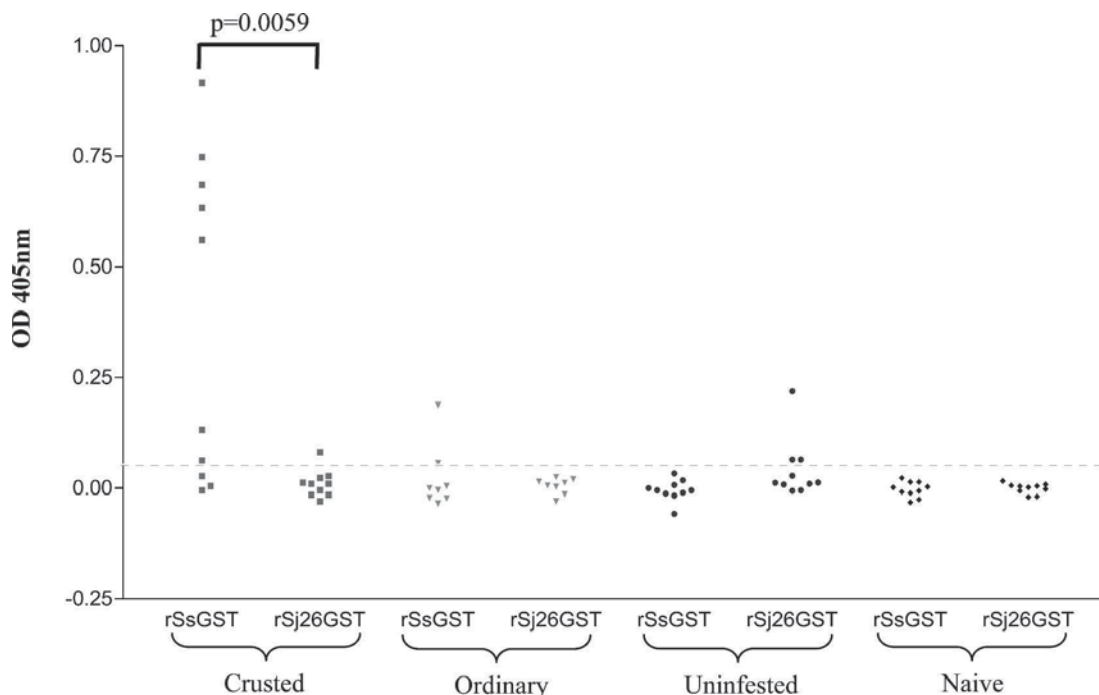


FIGURE 2. Specific IgE levels for rSsGST01 and rSj26GST in crusted, ordinary, uninfested, and naive sera, determined using ELISA.

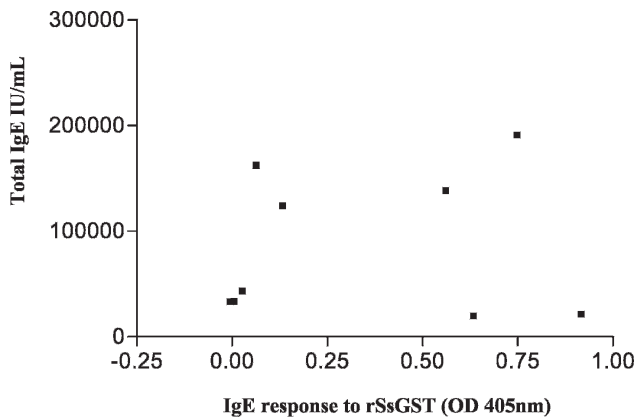


FIGURE 3. The relationship between specific IgE level for rSsGST01 determined using ELISA, and total serum IgE in individual patients.

was no significant difference in IgG binding to rSsGST01 between the crusted, ordinary, and uninfested sera groups, as determined by the Mann-Whitney test. Interestingly however, the uninfested sera again demonstrated a significantly higher response to the rSj26GST than to the rSsGST01 ($P = 0.0039$) using the Wilcoxon matched pairs test. This trend was also observed in the ordinary sera, although it was not found to be significant ($P = 0.0781$) (Figure 4a).

To determine if this was a result of antigenic cross-reactivity, the IgG response was then compared between uninfested and naive sera (Figure 4b). The higher IgG responses to both rSsGST01 and rSj26GST seen in the crusted, ordinary and uninfested groups were not seen in the naive group. The response to rSj26GST and rSsGST01 in the naive group was significantly lower than in the uninfested sera ($P = 0.0001$, $P = 0.0068$ respectively). There was no significant difference between the response to rSsGST01 and rSj26GST within the naive group ($P = 0.846$).

IgG4 reactivity of human sera to rSsGST01 and rSj26GST. The IgG4 response to rSsGST01 and rSj26GST (Figure 5) showed a similar pattern to the IgE response. The IgG4 response to rSsGST01 in the crusted sera was significantly higher than the ordinary ($P = 0.0266$) and uninfested ($P = 0.0021$) sera. Again, the crusted sera response to rSsGST01 compared with rSj26GST was significantly higher ($P = 0.0059$). However, no significant correlation was found between the paired IgG4 and IgE responses of individual crusted scabies patients to rSsGST01 using nonparametric (Spearman) correlation analysis (data not shown).

DISCUSSION

Scabies and house dust mite GSTs. Analysis of the primary amino acid sequences of the glutathione transferases identified, indicates that the Yv4001A08, Yv5004H11, and Yv5001D03 are likely to encode catalytically active mu class GST enzymes, whereas Yv4019D08, Yv4022A06, and Yv5001F03 are likely to encode catalytically active enzymes most closely related to the delta and epsilon GST of insects. In a parallel EST study on *D. pteronyssinus*, we have found a previously unrecognized mu class GST gene. As the published sequence of *Der p 8* is 97% identical to Dp7019C10, it

is not clear if this represents gene duplication or whether they are allelic forms of the same gene. However, Dp7002H05 is clearly a different gene. It is not obvious why the corresponding protein has not been observed in previous immunologic or protein based studies, as seven clones of this sequence were encountered compared with two of *Der p 8*. In addition, we have identified a novel *D. pteronyssinus* sequence most closely related to the delta and epsilon GST of insects.

The Acari may well have a unique complement of GST classes as we have identified both mu class GSTs, which are found in mammals but not insects, and a delta-like class, similar to the delta class of insects. In insects, the assignment of GSTs to a particular class can be influenced by the location of the gene within the genome.⁴⁰ At present, it is unknown how many classes of GSTs are represented in Acari and whether the genomic organisation of the genes will play a role in their classification.

Reaction of patient sera with rSsGST01. SsGST01, the protein that is encoded by the *S. scabiei* sequence Yv4001A08, has been identified as a major *S. scabiei* antigen. It is recognized by IgG from both crusted and ordinary scabies patients and additionally by patients with a previous history of scabies. Importantly it also appears to be a significant allergen of crusted scabies patients. SsGST01 does not seem to be recognized by individuals with no known history of scabies. The ability of rSsGST01 to bind to immobilized glutathione suggests that it is expressed in its native conformation in *E. coli*, as is well established for rSj26GST. This has proved to be an important issue with some house dust mite allergens, for example the group 1 allergen *Der p 1*.^{41,42}

High response to rSj26GST. Surprisingly, we observed a high IgG response to the rSj26GST in all scabies patient groups. This may indicate a cross-reaction between the rSj26GST and the rSsGST01 as observed with polyclonal rabbit antisera (data not shown) or a cross-reaction of the rSj26GST with other parasite GSTs that these patient groups may have been exposed to. The patients who have experienced infestation with *S. scabiei* are also likely to have been exposed to higher loads of unrelated parasites than the naive controls. It is probable that some or all patients may have been exposed to environmental schistosomes such as the larval form of *Austrobilharzia terrigalensis*, which occurs in northern Australian waterways, and causes a commonly recognized dermatitis associated with exposure to salt water.⁴³ It can infest humans causing "swimmers itch" but cannot be transmitted by humans.

Reaction of antibodies with SsGST01. There was no correlation between total IgE and SsGST01-specific IgE in the sera from crusted scabies patients. It is well documented that IgE levels are greatly elevated in crusted scabies.³⁹ Although on admittance to Royal Darwin Hospital, crusted scabies patients were not screened for the presence of active infections with helminth and/or protozoan parasites, all would have been exposed to *Trichurus trichiura*, as this is endemic in remote communities, and all would likely have had hookworm (*Ancylostoma duodenale*) as children. In general, all crusted and ordinary scabies patient would have received albendazole for worm therapy on many occasions. A small number had concurrent sepsis requiring antibiotics and the majority had *Trichophyton rubrum* (tinea) at some stage. There is also no current evidence of any overt immune complex disease, although an overrepresentation of antinuclear

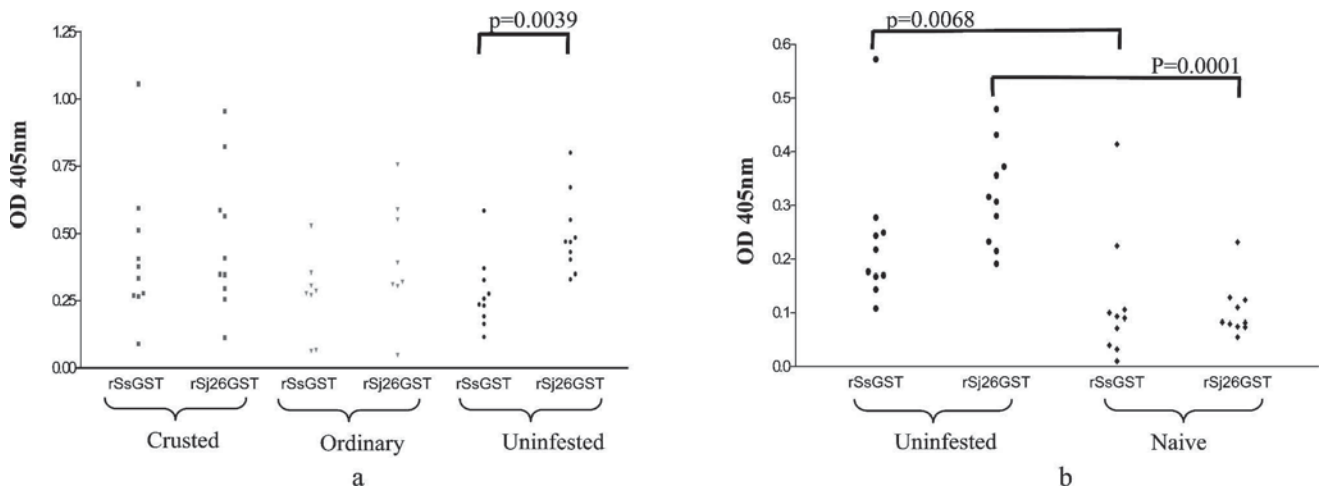


FIGURE 4. Specific IgG levels for rSsGST01 and rSj26GST in (a) crusted, ordinary, and uninfested sera and (b) uninfested and naive sera, determined using ELISA.

antibodies in these patients, with high titers recorded in 13% of 78 patients, has been documented.⁵ Sera from these crusted scabies patients reacted more strongly and with more antigens than did sera from ordinary scabies patients when tested against whole scabies mite antigen extracts.⁹ Further studies with additional cloned antigens will be required to determine to what extent the total IgE response is specific rather than non specific.

In *S. mansoni* infection, high levels of IgE were associated with resistance, whereas high levels of IgG4 antibodies were associated with increased susceptibility.⁴⁴ IgG4 is thought to compete with IgE for the same parasite epitopes in both schistosomiasis and filariasis.⁴⁵ The pattern of IgG4 reactivity observed here, like that of IgE, was much greater in the crusted scabies patients. However as removal of IgG by protein G did not change the observed IgE levels we have no evidence for such competition, suggesting in this case differences in epitope specificity. Further, pairwise comparisons revealed no correlation in IgG4 and IgE in individuals.

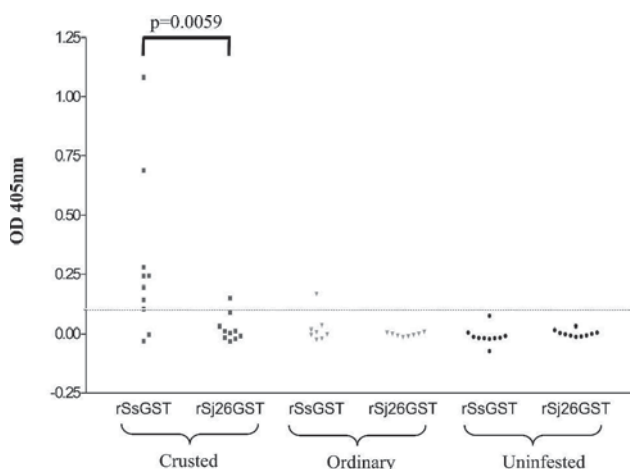


FIGURE 5. Specific IgG4 levels for rSsGST01 and rSj26GST in crusted, ordinary, uninfested, and naive sera determined using ELISA.

Research over the past decade has established that there is a fundamental skewing of immune responses in allergic diseases towards a Th2-phenotype. Although the Th1/Th2 model may be an oversimplification, we have hypothesised previously that in ordinary scabies the basal and antigen-stimulated cytokine response will resemble a Th1 response, and in crusted scabies a Th2 response.³⁹ In the Northern Territory, patients with crusted scabies are noted to have extremely high serum levels of total IgE and IgG.³⁹ Allergen-specific IgE plays a key role in the pathogenesis of allergic disorders. This IgE response is usually accompanied by production of IgG4.⁴⁶ However, the regulation of IgG4 responses and the function of these responses in the pathogenesis of allergy have not been fully characterized.⁴⁷ Human Th2 cytokines (interleukins 4 and 13) induce co-expression of IgE and IgG4 through sequential switching.⁴⁷ Baseline studies on cytokine production on mRNA obtained from PBMC collected from 8 crusted scabies patients and 2 uninfested controls indicated a statistically significant elevation of IL-4 in crusted scabies compared with controls.³⁹ The recognition that expression of IgG4 is IL-4-dependent and is an intermediate step in sequential switching from IgM to IgE now makes it important to understand how the two isotypes are regulated in crusted scabies and to ascertain the clinical effect of selectively boosting IgG4 over IgE. The ultimate goal would be to apply allergen specific immunotherapy for immunomodulatory control of crusted scabies.

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REFERENCES

1. Brook I, 1995. Microbiology of secondary bacterial infection in scabies lesions. *J Clin Microbiol* 33: 2139–2140.
2. Currie BJ, Carapetis J, 2000. Skin infections and infestations in Aboriginal communities in northern Australia *Australas J Dermatol* 41: 139–143.
3. McDonald M, Currie BJ, Carapetis JR, 2004. Acute rheumatic fever: a chink in the chain that links the heart to the throat? *Lancet Infect Dis* 4: 240–245.
4. Taplin D, Meinking TL, Chen JA, Sanchez R, 1990. Comparison of crotamiton 10% cream (eurax) and permethrin 5% cream (elimite) for the treatment of scabies in children. *Pediatr Dermatol* 7: 67–73.
5. Roberts LJ, Huffam S, Walton S, Currie B, 2005. Crusted scabies: clinical and immunological findings in seventy-eight patients and a review of the literature. *J Infect* 50: 375–381.
6. Mellanby K, 1944. The development of symptoms, parasitic infection and immunity in human scabies. *Parasitology* 35: 197–206.
7. Arlian LG, Morgan MS, Vyszenski-Moher DL, Stemmer BL, 1994. *Sarcoptes scabiei*: The circulating antibody response and induced immunity to scabies. *Exp Parasitol* 78: 37–50.
8. Arlian LG, Rapp CM, Vyszenski-Moher DL, Morgan MS, 1994. *Sarcoptes scabiei*: Histopathological changes associated with acquisition and expression of host immunity to scabies. *Exp Parasitol* 78: 51–63.
9. Arlian LG, Morgan MS, Estes SA, Walton SF, Kemp DJ, Currie BJ, 2004. Circulating IgE in patients with ordinary and crusted scabies. *J Med Entomol* 41: 74–77.
10. Mattsson JG, Ljunggren EL, Bergstrom K, 2001. Paramyosin from the parasitic mite *Sarcoptes scabiei*: cDNA cloning and heterologous expression. *Parasitology* 122: 555–562.
11. Fischer K, Holt DC, Harumal P, Currie BJ, Walton SF, Kemp DJ, 2003. Generation and characterisation of cDNA clones from *Sarcoptes scabiei* var. *hominis* for an expressed sequence tag library: identification of homologues of house dust mite allergens. *Am J Trop Med Hyg* 68: 61–64.
12. Harumal P, Morgan MS, Walton SF, Holt DC, Rode J, Arlian LG, Currie BJ, Kemp DJ, 2003. 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. *Am J Trop Med Hyg* 68: 54–60.
13. Pettersson EU, Ljunggren EL, Morrison DA, Mattsson JG, 2005. Functional analysis and localisation of a delta-class glutathione S-transferase from *Sarcoptes scabiei*. *Int J Parasitol* 35: 39–48.
14. Holt DC, Fischer K, Allen GE, Wilson D, Wilson P, Slade R, Currie BJ, Walton SF, Kemp DJ, 2003. Mechanisms for a novel immune evasion strategy in the scabies mite *Sarcoptes scabiei*: A multigene family of inactivated serine proteases. *J Invest Dermatol* 121: 1419–1424.
15. Holt DC, Fischer K, Pizzutto SJ, Currie BJ, Walton SF, Kemp DJ, 2004. A multigene family of inactivated cysteine proteases in *Sarcoptes scabiei*. *J Invest Dermatol* 123: 240–241.
16. O'Neill GM, Donovan GR, Baldo BA, 1994. Cloning and characterization of a major allergen of the house dust mite *Dermatophagoides pteronyssinus*, homologous with glutathione S-transferase. *Biochim Biophys Acta* 1219: 521–528.
17. Rosa de Lima MF, Sanchez Ferreira CA, Joaquim de Freitas DR, Valenzuela JG, Masuda A, 2002. Cloning and partial characterization of a *Boophilus microplus* (Acari: Ixodidae) glutathione S-transferase. *Insect Biochem Mol Biol* 32: 747–754.
18. Sommer A, Rickert R, Fischer P, Steinhart H, Walter RD, Liebau E, 2003. A dominant role for extracellular glutathione S-transferase from *Onchocerca volvulus* is the production of prostaglandin D2. *Infect Immun* 71: 3603–3606.
19. Pickett CB, Lu AY, 1989. Glutathione S-transferases: gene structure, regulation, and biological function. *Annu Rev Biochem* 58: 743–764.
20. Armstrong RN, 1991. Glutathione S-transferases: reaction mechanism, structure, and function. *Chem Res Toxicol* 4: 131–140.
21. Wilce MC, Parker MW, 1994. Structure and function of glutathione S-transferases. *Biochim Biophys Acta* 1205: 1–18.
22. Hayes JD, Pulford DJ, 1995. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol* 30: 445–600.
23. Favalaro B, Tamburro A, Angelucci S, Luca AD, Melino S, di Ilio C, Rotilio D, 1998. Molecular cloning, expression and site-directed mutagenesis of glutathione S-transferase from *Ochrobactrum anthropi*. *Biochem J* 335: 573–579.
24. Sheehan D, Meade G, Foley VM, Dowd CA, 2001. Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. *Biochem J* 360: 1–16.
25. Arruda LK, Vailes LD, Platts-Mills TA, Hayden ML, Chapman MD, 1997. Induction of IgE antibody responses by glutathione S-transferase from the German cockroach (*Blattella germanica*). *J Biol Chem* 272: 20907–20912.
26. O'Neill GM, Donovan GR, Baldo BA, 1995. Glutathione S-transferase a major allergen of the house dust mite, *Dermatophagoides pteronyssinus*. *Immunol Lett* 48: 103–107.
27. Spithill TW, Piedrafita D, Smooker PM, 1997. Immunological approaches for the control of fasciolosis. *Int J Parasitol* 27: 1221–1235.
28. Riveau G, Poulain-Godefroy OP, Dupre L, Remoue F, Mielcarek N, Loch C, Capron A, 1998. Glutathione S-transferases of 28kDa as major vaccine candidates against schistosomiasis. *Mem Inst Oswaldo Cruz* 93 (Suppl 1): 87–94.
29. Sexton JL, Wilce MC, Colin T, Wijffels GL, Salvatore L, Feil S, Parker MW, Spithill TW, Morrison CA, 1994. Vaccination of sheep against *Fasciola hepatica* with glutathione S-transferase. Identification and mapping of antibody epitopes on a three-dimensional model of the antigen. *J Immunol* 152: 1861–1872.
30. He H, Chen AC, Davey RB, Ivie GW, George JE, 1999. Characterization and molecular cloning of a glutathione S-transferase gene from the tick, *Boophilus microplus* (Acari: Ixodidae). *Insect Biochem Mol Biol* 29: 737–743.
31. Lee AJ, Huntley J, Van den Broek A, Coates D, Isaac RE, 2002. Expression and characterisation of a *Psoroptes ovis* glutathione S-transferase. *Vet Parasitol* 105: 49–63.
32. Fischer K, Holt DC, Wilson P, Davis J, Hewitt V, Johnson M, McGrath A, Currie BJ, Walton SF, Kemp DJ, 2003. Normalization of a cDNA library cloned in λZAP by a long PCR and cDNA reassociation procedure. *Biotechniques* 34: 250–254.
33. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ, 1990. Basic local alignment search tool. *J Mol Biol* 215: 403–410.
34. Thompson JD, Higgins DG, Gibson TJ, 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22: 4673–4680.
35. Felsenstein J, 1989. PHYLIP—Phylogeny Inference Package (Version 3.2). *Cladistics* 5: 164–166.
36. Towbin H, Staehelin T, Gordon J, 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Nat Acad Sci USA* 76: 4350–4354.
37. Burnette W, 1981. “Western blotting”: Electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gel to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal Biochem* 112: 195–203.
38. Allocati N, Casalone E, Masulli M, Ceccarelli I, Carletti E, Parker MW, Di Ilio C, 1999. Functional analysis of the evolutionarily conserved proline 53 residue in *Proteus mirabilis* glutathione transferase B1-1. *FEBS Lett* 445: 347–350.
39. Walton SF, Holt DC, Currie BJ, Kemp DJ, 2004. Scabies: new future for a neglected disease. *Adv Parasitol* 57: 309–376.

40. Ding Y, Ortelli F, Rossiter LC, Hemingway J, Ranson H, 2003. The *Anopheles gambiae* glutathione transferase supergene family: annotation, phylogeny and expression profiles. *BMC Genomics* 4: 35.
41. Gough L, Schulz O, Sewell HF, Shakib F, 1999. The cysteine protease activity of the major dust mite allergen Der p 1 selectively enhances the immunoglobulin E antibody response. *J Exp Med* 190: 1897–1902.
42. Yasuhara T, Takai T, Yuuki T, Okudaira H, Okumura Y, 2001. Biologically active recombinant forms of a major house dust mite group 1 allergen Der f 1 with full activities of both cysteine protease and IgE binding. *Clin Exp Allergy* 31: 116–124.
43. Stevenson WJ, Hughes KL, 1988. *Synopsis of Zoonoses in Australia*. Canberra: Commonwealth Department of Community Services.
44. Capron A, Dessaint JP, Capron M, Pierce RJ, 1992. Vaccine strategies against schistosomiasis. *Mem Inst Oswaldo Cruz* 87 (Suppl 5): 1–9.
45. Garraud O, Perraut R, Riveau F, Nutman T, 2003. Class and subclass selection in parasite-specific antibody responses. *Trends Parasitol* 19: 300–304.
46. Aalberse RC, Van Milligen F, Tan KY, Stapel SO, 1993. Allergen-specific IgG4 in atopic disease. *Allergy* 48: 559–569.
47. Stern D, Eder W, Tebow G, Lohman IC, Soprana E, Braun-Fahrlander C, Riedler J, Nowak D, Von Mutius E, Halonen M, Vercelli D, 2004. Rethinking Th2 antibody responses and allergic sensitization. *Novartis Found Symp* 257: 25–37.