



Research paper

Molecular characterization and allergenicity potential of triosephosphate isomerase from *Sarcoptes scabiei*

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ABSTRACT

Scabies is an allergic skin disease that affects millions of mammals worldwide, including humans. It is a neglected tropical disease that represents a significant public health threat, particularly in economically disadvantaged populations. An effective vaccine is not currently available, and the exact mode of pathogenesis remains unclear. Herein, we identified, cloned and recombinantly expressed triosephosphate isomerase from *Sarcoptes scabiei* (*S. scabiei*). Immunohistochemical analyses showed that *S. scabiei* triosephosphate isomerase (Ss-TIM) is localized in the legs and chewing mouthparts of mites, and in infected rabbit skin (keratinized skin and embedded mites). Intradermal skin tests of rabbits injected with recombinant *S. scabiei* triosephosphate isomerase (rSs-TIM) revealed a flare, erythema and wheal reaction. These findings suggest that Ss-TIM may contribute to host invasion and induce an allergic response in the host.

1. Introduction

Scabies is a skin disease that affects millions of humans, wild and domestic mammals worldwide, and is most prevalent in socio-economically disadvantaged populations in tropical regions (Foster et al., 2015; Grahofer et al., 2018; Karimkhani et al., 2017; Kriechbaum et al., 2018; Pence and Ueckermann, 2002; Steer et al., 2009). It is caused by the itch mite, *Sarcoptes scabiei*, which burrows under the skin causing intense itching leading to scratching, and may result in complex secondary infections through mite-associated bacterial pathogens (Currie and McCarthy, 2010; Das et al., 2017; Espinosa et al., 2017; Prakash et al., 2017). There is no available vaccine against scabies, and the feasibility and affordability of vaccine development is doubtful (Liu et al., 2014). Scabies treatment currently depends on a few broad-spectrum anti-parasitic drugs such as ivermectin (Hamel et al., 2015; Hardy et al., 2017). *S. scabiei* undergoes several developmental stages, namely egg, larva, protonymph, tritonymph and adult, and its entire life-cycle occurs within the host (Arlian and Morgan, 2017; Hicks and Elston, 2009). The characteristics of *S. scabies* make it difficult to

continuously culture *in vitro*, and existing molecular research is limited (He et al., 2017a).

Triosephosphate isomerase (TIM) is an enzyme that catalyzes the reversible interconversion of dihydroxyacetone phosphate and the triose phosphate glyceraldehyde 3-phosphate. The enzyme is placed at a non-linear step in the catabolic process to enhance the efficiency of glycolysis without producing pyruvate (Knowles, 1991; Roland et al., 2015; Yang et al., 2017a). TIMs belong to the ($\beta\alpha$)₈-barrel class of proteins in which eight parallel β -strands form the central core of the barrel, which is surrounded by eight α -helices. TIM plays a crucial role in the net production of ATP in glycolysis, which represents a potential target for drug design against glycolytic pathogens (Kumar et al., 2012). TIMs are widely distributed in all organisms (bacteria, fungi, plants and mammals) and have been linked to worsening the condition of allergy sufferers (Anuar et al., 2014; Dai et al., 2014; Hewitson et al., 2014; Yang et al., 2017a; Zhou et al., 2015). TIM can also be excreted (Mulvenna et al., 2010; Zheng et al., 2011), and parasite excretory proteins are reported to be involved in host-parasite interactions, mite invasion, host immune regulation and immune evasion processes (Geary et al.,

Abbreviations: TIM, Triosephosphate isomerase; *Sarcoptes scabiei*, *S. scabiei*; Ss-TIM, *Sarcoptes scabiei*; TIMrSs-TIM, recombinant *Sarcoptes scabiei* TIM; ORF, Open reading frame; SDS-PAGE, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBS, Phosphate-buffered saline; TBS, Tris-buffered saline; PBST, PBS containing 0.05% Tween 20; TBST, TBS containing 0.05% Tween 20; TMB, Tetramethylbenzidine; OD, Optical density; SD, Standard deviations; ELISA, Enzyme-linked immunosorbent assay

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2012; Hewitson et al., 2008). Additionally, transcriptomic (He et al., 2017a), proteomic (Morgan et al., 2016) and genomic (Rider et al., 2015) analyses of *S. scabiei canis* demonstrated that TIM shares high homology with the group 25 allergen from house dust mite (HDM), and mite allergens can promote allergic reactions and inflammation, including dermatitis, rhinitis and asthma (Aalberse, 2000; Arlian, 2002; Lee et al., 1999; Tournoy et al., 2006). Therefore, determining the distribution of Ss-TIM in infected skin and *S. scabiei* mites may contribute to our understanding of host immune responses and mite pathogenesis. The aim of the present study was to determine the molecular characteristics of the Ss-TIM protein and to explore its role in scabies pathogenesis.

2. Materials and methods

2.1. Mites and animals

S. scabiei larvae, nymphs and adults were provided by the Department of Parasitology in Sichuan Agricultural University, and nine female New Zealand white rabbits (8-week-old) were provided by the Laboratory Animal Center of Sichuan Agricultural University.

2.2. Sequence analysis of Ss-TIM

The sequence of Ss-TIM was obtained from our *S. scabiei* transcriptome data (NCBI Bio Project ID: PRJNA320671) (He et al., 2017a). The ExPASy Proteomics Server (<http://web.expasy.org/protparam/>) was used to predict the isoelectric point. Open reading frame (ORF) Finder (<http://www.ncbi.nlm.nih.gov/orffinder/>) was used to determine the open reading frame. The SignalP 4.1 Server (<http://www.cbs.dtu.dk/services/SignalP/>) was used to predict the Signal peptides. DNAMAN 3.0 (Lynnon Biosoft, Quebec, Canada) and the online BLASTp tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) were used to perform multiple sequence alignment. The YASPIN program (<http://www.ibi.vu.nl/programs/yaspinwww/>) was used to predict secondary structure, and MEGA 5.1 (<http://www.megasoftware.net/>) was used to create the phylogenetic tree by the neighbor-joining (NJ) method with related sequences from other species using 1000 bootstrap replicates and Poisson correction of gaps/missing data.

2.3. Expression and purification of rSs-TIM

An RNA Extraction Kit (Cowan Biotech, China) was used to isolate total RNA from mites according to the manufacturer's instructions. RNA quality and quantity were assessed using a Nanodrop 2000 spectrophotometer (Thermo, USA) and agarose gel electrophoresis (1.5%). The RevertAi First Strand cDNA Synthesis Kit (Thermo, USA) was used to reverse-transcribe 1 µg of total RNA to cDNA according to the manufacturer's instructions, and the product was stored at -80°C . The region encoding Ss-TIM was amplified by PCR using forward primer 5'-CGCGGATCGGTTCCAAGCATTTACCTTTCT-3' and reverse primer 5'-CCGCTCGAGGTGTGGAATTTTATTCTGTGATG-3'. Cycling parameters were 94°C for 5 min, followed by 29 cycles of 94°C for 50 s, 60°C for 50 s, 72°C for 1 min, and a final elongation step at 72°C for 10 min. PCR products were separated and cloned into the PMD19-T vector (TaKaRa, China) and subcloned into the pET-32a(+) vector (Novagen, USA) using the *Bam*HI and *Xho*I restriction sites (underlined in the above primers). The recombinant TIM-pET32a(+) plasmid was transformed into *E. coli* BL21 (DE3) cells (Invitrogen, USA), and 1 mM isopropyl β -D-1-thiogalactopyranoside was used to induce expression of cultured cells. Recombinant Ss-TIM (rSs-TIM) was purified by chromatography with a Ni²⁺-NTA His-tag affinity kit (Bio-Rad, USA) according to the manufacturer's instructions. rSs-TIM was analyzed for purity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 12% gel, and subsequently with a bicinchoninic

acid protein assay kit (Pierce, USA) to estimate protein concentration.

2.4. Serum preparation

Serum positive controls for *S. scabiei*, *Psoroptes cuniculi*, *Taenia pisiformis*, *Eimeria* spp. and serum negative controls from healthy rabbits were provided by the Department of Parasitology, College of Veterinary Medicine, Sichuan Agricultural University, China. Serum positive controls for *S. scabiei*, *Psoroptes cuniculi*, *Taenia pisiformis* and *Eimeria* spp. were prepared from pooled rabbits serum (12 rabbits for *S. scabiei* and six rabbits for the others). For the preparation of anti-serum, rSs-TIM was emulsified with Saponin adjuvant (Sigma, USA) and 200 µg of recombinant protein was injected subcutaneously. Subsequent subcutaneous injections of 100 µg of rSs-TIM protein were performed on day 7 and day 14, and injection of 200 µg of recombinant protein was used for the final immunization on day 21. Anti-sera from rabbits ($n = 3$) was collected 1 week after the final immunization. The antibody titer was investigated by ELISA (Wiederschain, 2009), and a Protein G-Sepharose column (Bio-Rad, USA) was used to isolate immunoglobulin G (IgG) from the anti-serum according to the manufacturer's instructions.

2.5. Identification of rSs-TIM

Total crude protein was extracted from mites using a total protein extraction kit (BestBio, Shanghai, China). Protein samples were boiled for 10 min in electrophoresis sample buffer, then separated by 12% SDS-PAGE. An electrophoretic transfer cell (Bio-Rad, USA) was used to transfer the protein onto a nitrocellulose membrane, and the membrane was incubated with blocking buffer (5% skim milk) for 2 h, followed by serum samples (diluted 1:100 in PBS) at 4°C overnight. After washing three times with TBS-Tween 20 (TBST), the membrane was incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody (1:3000; Boster, China) for 2 h. The Enhanced HRP-DAB Chromogenic Substrate Kit (Tiangen, China) was used to visualize the protein signal according to the manufacturer's instructions.

2.6. Indirect ELISA

The serodiagnostic potential of rSs-TIM was evaluated by indirect ELISA. This was performed essentially as previously described (He et al., 2017c; Wiederschain, 2009). Polystyrene 96-well microtiter plates (Invitrogen, USA) were coated with rSs-TIM diluted in 0.1 M carbonate buffer (pH 9.6) overnight at 4°C , then incubated with 100 µL blocking buffer (5% skim milk in PBS) for 90 min at 37°C after washing three times with PBST. Serial two-fold diluted serum samples were added, followed by goat anti-rabbit IgG-HRP conjugate (100 µL, 1:3000; Earth Ox, USA). The antibody was then detected using 100 µL of 3,3',5,5'-tetramethylbenzidine (TMB; Tiangen, China). The specificity and sensitivity of the rSs-TIM indirect ELISA (iELISA) were evaluated using negative and positive sera collected as described in Section 2.4. The percentage analytical sensitivity was calculated as (iELISA positive $\times 100$) / true positive, and the percentage analytical specificity was calculated as (iELISA negative $\times 100$) / true negative (Varghese et al., 2012; Yang et al., 2013).

2.7. Immunohistochemical analyses

Mites provided by the Department of Parasitology in Sichuan Agricultural University, and were collected and stored in liquid nitrogen after 12 h of starvation. New Zealand white rabbits ($n = 6$) were anesthetized with ketamine and then euthanized. Foot skin sampling was then undertaken using an annular skin sampler with a diameter of 7 mm. All samples were fixed with 1% molten agarose prior to solidification in paraffin wax. Embedded samples were cut into 5 µm sections using a rotary microtome. Immunohistochemical assays were

performed as previously described (Zheng et al., 2016). Briefly, sections were rehydrated and rinsed in PBS, then incubated with purified rabbit anti- Ss-TIM IgG (diluted 1:100 in PBS) overnight at 4 °C. Sections were then incubated with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (diluted 1:100 in 0.1% Evans blue; H + L; AMRESCO, Texas, USA) for 1 h at 37 °C in the dark. Sections were washed with PBS and then imaged with a fluorescence microscope (Olympus, Japan) (He et al., 2017b). Naïve rabbit sera were used as negative controls instead of specific antibodies. To complement the results of fluorescent immunohistochemistry, H&E staining was performed as a control.

2.8. Intradermal skin test

IgE ELISA and IgE dot blotting could not be performed due to the lack of an effective secondary anti-rabbit IgE antibody. Thus, intradermal skin tests were used to preliminarily investigate the allergenic activity of rSs-TIM. Three New Zealand White rabbits were injected with purified rSs-TIM (400 µg in 0.1 mL PBS) subcutaneously, and 4 mg/mL histamine (0.1 mL) served as a positive control, while purified pET-32a (+) empty expression vector (400 µg in 0.1 mL PBS), 0.9% physiological saline (0.1 mL) and PBS (0.1 mL) served as negative controls. Positive and negative controls were injected three times on the back of each rabbit, and skin reactions were observed and defined as positive when the presence of a wheal and erythema was observed.

3. Results

3.1. Sequence analysis of *S. scabiei* TIM

The Ss-TIM cDNA contains a 504 bp ORF encoding a putative protein of 167 amino acids with a predicted molecular mass of 18.3 kDa and an isoelectric point of 5.4 without a signal peptide. Multiple alignment of TIMs revealed that the amino acid sequence of Ss-TIM shares 89% and 88% identity with the Der f group 25 allergen from

Dermatophagoides farinae, and the group 25 allergen-like protein (triose-phosphate isomerase) from *Euroglyphus maynei*, respectively (Fig. 1). The Ss-TIM amino acid sequence also shares 73–77% overall identity with triose-phosphate isomerase from more than 100 other species including *Parasteatoda tepidariorum*, *Rhagoletis zephyria*, *Lepisosteus osseus*, *Ophiophagus hannah*, *Amia calva* and *Canis lupus familiaris* (Fig. 1). Phylogenetic analysis showed that the TIMs of 20 species mainly fall into two separate arthropod and helminth branches, with *S. scabiei* TIM in the arthropod branch along with the enzyme from *D. farinae* and *E. maynei* (Fig. 2).

3.2. Expression and characterization of rSs-TIM

The cDNA encoding Ss-TIM was cloned into the pET32a (+) expression vector (Invitrogen, China) and successfully expressed in *E. coli* BL21 (DE3). The molecular weight (MW) of rSs-TIM was approximately 37 kDa (including the His tag; Fig. 2), similar to the predicted MW. The rSs-TIM protein was purified by Ni-chelating column chromatography and subjected to SDS-PAGE (Fig. 2). Immunoblotting using *S. scabiei*-positive rabbit serum and anti-rSs-TIM rabbit serum revealed a single band at approximately 37 kDa (including the His tag), indicating good antigenicity for rSs-TIM. No signal band was detected using naïve rabbit serum. When total crude proteins were probed with anti-rSs-TIM rabbit serum, a ~18 kDa protein band was observed (Fig. 3).

3.3. Establishment of indirect ELISA (iELISA)

The optimal serum dilution and optimal concentration of the rSs-TIM antigen for ELISA were found to be 1:640 and 1 µg/well, respectively. Negative serum was also analyzed using the optimized conditions. The mean OD₄₅₀ value was 0.253, and the standard deviation was 0.052, which gave a cut-off value of 0.409 (mean + 3SD) (Crowther and Crowther, 2000). Inter-assay coefficients of variation (CV) ranged from 2.30% to 5.64% (mean = 3.97%), while intra-assay CVs ranged



Fig. 1. Sequence alignment of *Sarcoptes scabiei* triose-phosphate isomerase (TIM). Alignment of the deduced amino acid sequence of *S. scabiei* TIM (Ss-TIM) with homologues from other species. The percentage homology of Ss-TIM with each TIM is shown at the end of the alignment. The predicted secondary structure of Ss-TIM is displayed above the alignment. Within regions of identity, the dark part indicates exactly matching bases, and the light color indicates partial matching. *Sarcoptes scabiei* TIM (Ss-TIM). GenBank accession numbers: *Dermatophagoides farinae* TIM (D. f-TIM; [AIO08860.1](#)), *Euroglyphus maynei* TIM (E. m-TIM; [OTF72634.1](#)), *Rhagoletis zephyria* TIM (R. z-TIM; [XP017478126.1](#)), *Wasmannia auropunctata* TIM (W. a-TIM; [XP011698775.1](#)), *Drosophila melanogaster* TIM (D. m-TIM; [NP788766.1](#)), *Dugesia japonica* TIM (D. j-TIM; [ABZ90134.1](#)), *Schistosoma turkestanicum* TIM (S. t-TIM; [AAZ57433.1](#)), *Hymenolepis microstoma* TIM (H. m-TIM; [CDS29962.1](#)), *Echinococcus granulosus* TIM (E. g-TIM; [CDS23836.1](#)), *Fasciola hepatica* TIM (F. h-TIM; [AGJ83762.1](#)), *Clonorchis sinensis* TIM (C. s-TIM; [GAA50994.1](#)), *Brugia malayi* TIM (B. m-TIM; [XP001897269.1](#)).

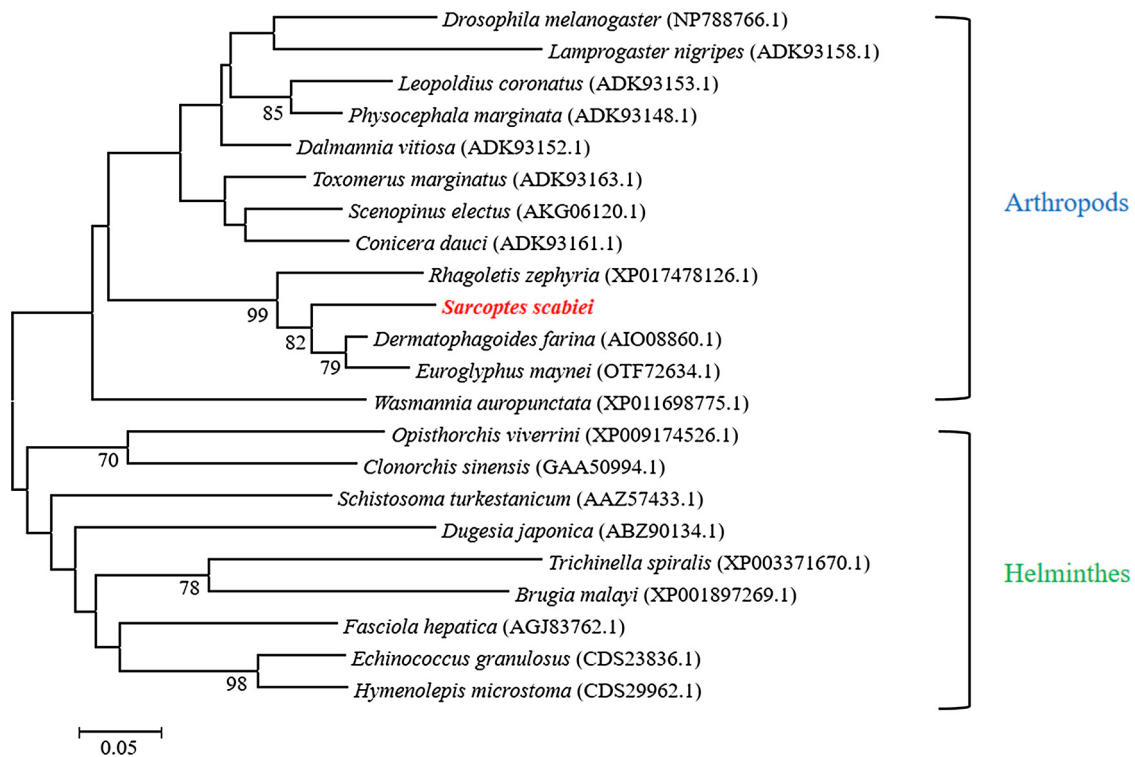


Fig. 2. Phylogenetic tree of Ss-TIM and enzymes from other species. The tree was constructed by the neighbor-joining (NJ) method in MEGA version 5. GenBank accession numbers: *Drosophila melanogaster* (NP788766.1), *Lamprogaster nigripes* (ADK93158.1), *Leopoldius coronatus* (ADK93153.1), *Physocephala marginata* (ADK93148.1), *Dalmannia vitiosa* (ADK93152.1), *Toxomerus marginatus* (ADK93163.1), *Scenopinus electus* (AKG06120.1), *Conicera dauci* (ADK93161.1), *Rhagoletis zephyria* (XP017478126.1), *Dermatophagoides farina* (AIO08860.1), *Euroglyphus maynei* (OTF72634.1), *Wasmannia auropunctata* (XP011698775.1), *Opisthorchis viverrini* (XP009174526.1), *Clonorchis sinensis* (GAA50994.1), *Schistosoma turkestanicum* (AAZ57433.1), *Dugesia japonica* (ABZ90134.1), *Trichinella spiralis* (XP003371670.1), *Brugia malayi* (XP001897269.1), *Fasciola hepatica* (AGJ83762.1), *Echinococcus granulosus* (CDS23836.1), *Hymenolepis microstoma* (CDS29962.1).

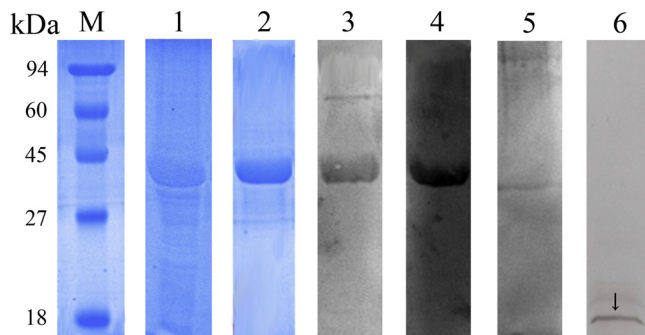


Fig. 3. Expression and identification of rSs-TIM. M, protein molecular weight markers; lane 1, non-purified rSs-TIM; lane 2, purified rSs-TIM; lane 3, purified rSs-TIM detected with *S. scabiei*-positive rabbit serum; lane 4, purified rSs-TIM detected with anti-rSs-TIM rabbit serum; lane 5, purified rSs-TIM detected with naïve rabbit serum; lane 6, total crude proteins detected with anti-rSs-TIM rabbit serum.

from 2.96% to 7.01% (mean = 4.99%). All CVs were < 10%, confirming repeatability and reproducibility.

Serum from 12 *S. scabiei*-positive rabbits, six *Psoroptes cuniculi*-positive rabbits, six *Eimeria* spp.-positive rabbits, and six *Taenia pisiformis*-positive rabbits were investigated by iELISA. Serum from nine *S. scabiei*-positive rabbits and serum from eight rabbits (four *Psoroptes cuniculi*-positive, three *Taenia pisiformis*-positive, and one *Eimeria* spp.-positive) tested positive, with an assay specificity of 44.4% (8/18) and sensitivity of 75% (9/12). Moreover, differences observed in the OD₄₅₀ values between negative and positive sera were statistically significant ($p < 0.05$; data not shown).

3.4. Immunolocalization

H&E staining results showed that scabies invaded the epidermis of host skin (Fig. 4). An immunofluorescence method was also used to determine the distribution of Ss-TIM, and fluorescence signals were observed in the legs and chewing mouthparts in starved mites (Fig. 5), and also in embedded mites and keratinized skin of infected rabbits. Negative controls did not generate a fluorescence signal (Fig. 6).

3.5. Intradermal skin test

Rabbits injected with purified rSs-TIM produced a wheal, erythema and flare reaction. PBS, 0.9% physiological saline, and empty expression vector induced no reaction, whereas a wheal reaction was induced by histamine (Fig. 7).

4. Discussion

Multiple alignment of TIMs revealed that the amino acid sequence of Ss-TIM is similar to TIM sequences from many other species, and phylogenetic analysis showed that Ss-TIM belongs to the same arthropod branch as TIMs from *D. farinae* and *E. maynei*. TIM is widely distributed in all organisms, suggesting that the enzyme has been highly conserved through evolution, which may explain the observed low specificity. Meanwhile, cross-reaction was observed for serum samples from rabbits infected with different parasites, which might also contribute to the low specificity.

Allergic reactions can be protective and lead to immunity, but allergy can also be harmful and lead to tissue damage and immunological hypersensitivity. Allergenicity refers to the ability of an antigen to induce allergic sensitization. Allergens induce immune responses in hosts

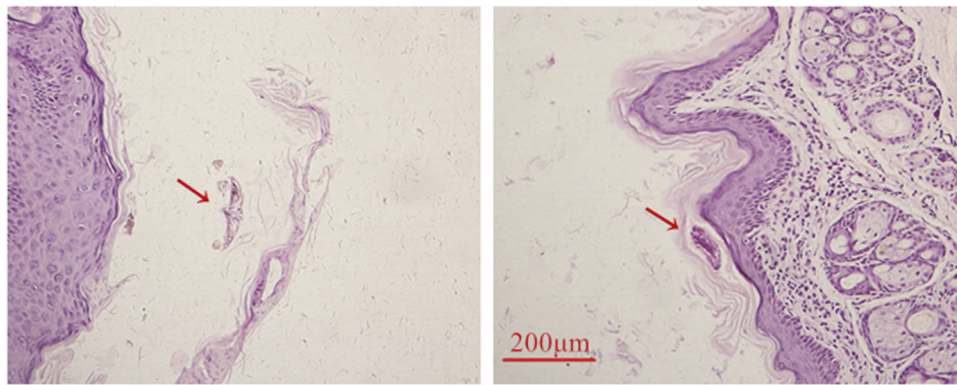


Fig. 4. Hematoxylin and eosin (H&E) staining of rabbit skin in *S. scabiei*-infected rabbit.

that can cause immunological hypersensitivity mediated by the IgE antibody particularly type I hypersensitivity (Chapman et al., 2007; Hales et al., 2006; Thepen et al., 1992; Tzolakis et al., 2018). The reactivity of an allergen-induced IgE antibody is one index used to define allergenicity, which indicates that the immune system has been transformed into an allergic state (Mizuma et al., 2016; Ogunlade et al., 2012; Thomas et al., 2010).

Scabies, caused by allergens, is a type of allergic skin disease. Most allergens from mites are proteases that promote allergic reactions and inflammation, such as asthma, dermatitis and rhinitis (Aalberse, 2000;

Arlian, 2002; Marsella and De Benedetto, 2017; Stave, 2018; Stewart and Robinson, 2003). Der p 2 and Der p 1 are house dust mite allergens from *D. pteronyssinus* that enhance allergic immune responses in the host by boosting the innate immune response and cleaving IgE receptors (Gough et al., 1902; Trompette et al., 2008). Allergens of cockroaches induce immunological responses by binding to either C-type lectin receptors or Toll-like receptors, thereby activating innate immune cells (Gao, 2012). These findings suggest that allergens from *S. scabiei* may be crucial in the pathogenesis of scabies. Previous studies showed that TIMs are also expressed in other arthropods, such as

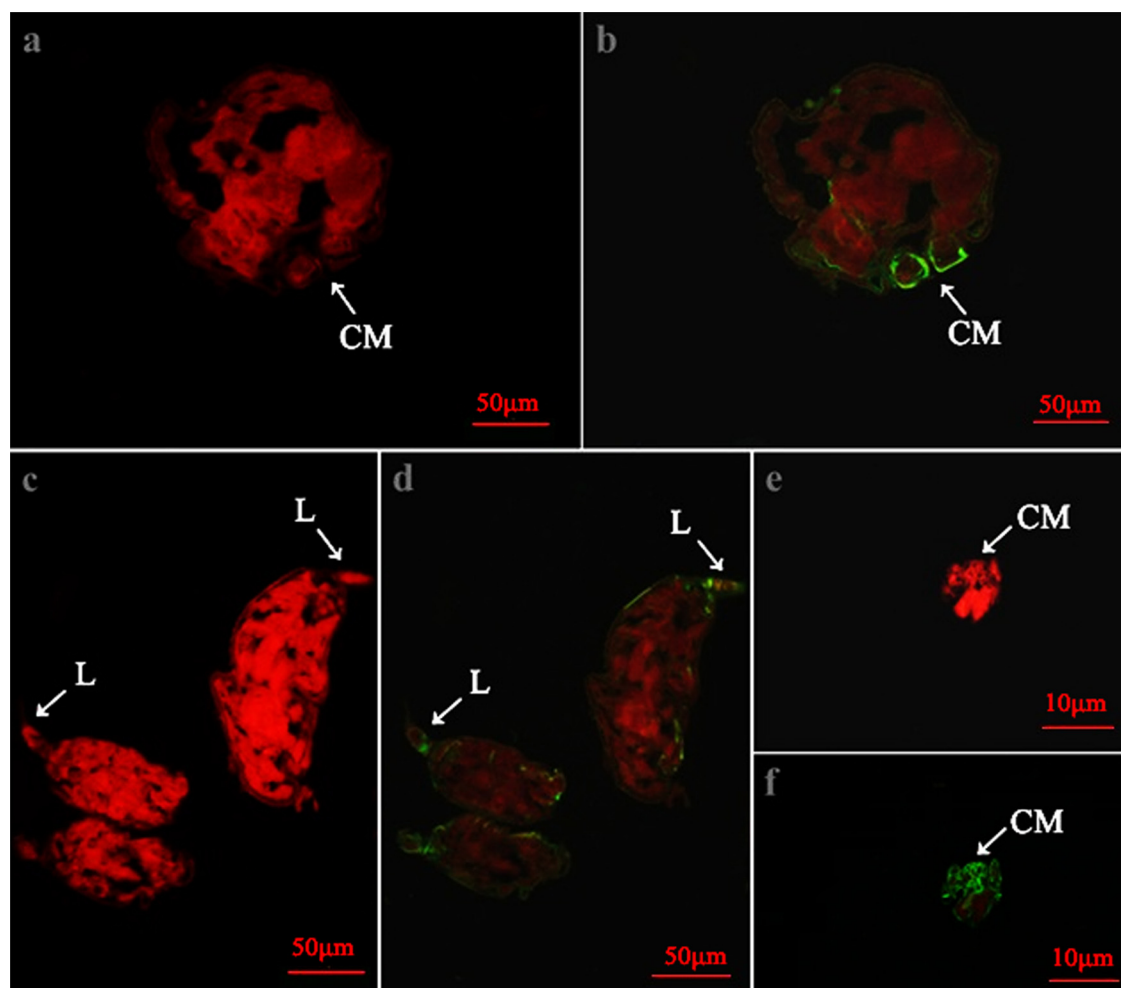


Fig. 5. Immunofluorescence localization of Ss-TIM in starved mites. a, c, e, Negative controls (staining with naïve rabbit serum as primary antibody); b, d, f, Staining with anti-TIM as the primary antibody. CM, chewing mouthparts; L, legs.

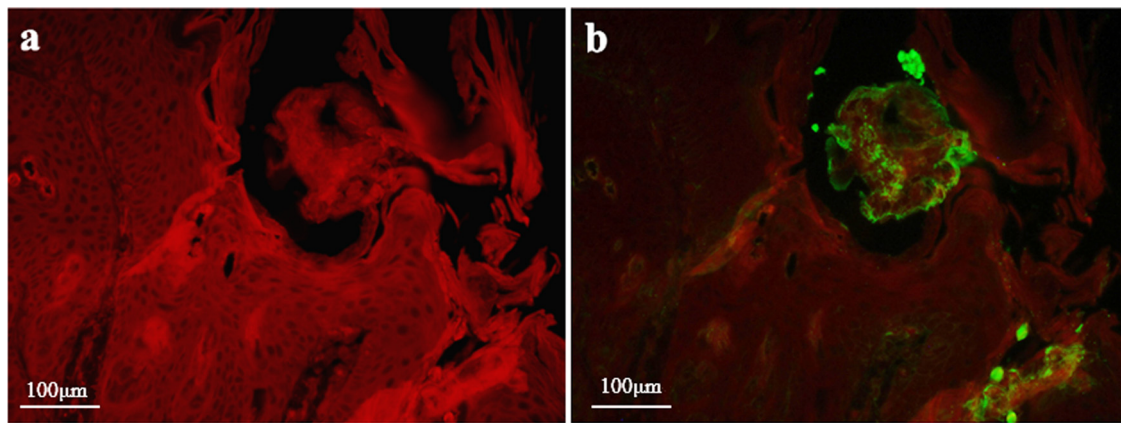


Fig. 6. Immunofluorescence localization of Ss-TIM in *S. scabiei*-infected rabbit skin. Ss-TIM is distributed in embedded mites and keratinized skin. a, Negative control (no primary antibody); b, Staining with anti-TIM as the primary antibody.

Rhipicephalus (Boophilus) microplus, *Apis mellifera ligustica* and *D. farina*, and TIMs have also been identified as an allergen in many species including *Octopus fangsiao*, *Blattella germanica*, *B. malayi* and *D. farina* (Hewitson et al., 2008; Hoppe et al., 2006; Lin et al., 2016; Moraes et al., 2011; Walter et al., 2011; Xu et al., 2009; Yang et al., 2017a; Yang et al., 2017b).

S. scabiei mites obtain nutrients from host epidermal cells and serum (Arlian et al., 2015; Casais et al., 2014; Hewitson et al., 2008). In starved mites, fluorescence signals showed that Ss-TIM is localized at the chewing mouthparts and legs of *S. scabiei* mites, and fluorescence signals were also detected in embedded mites and keratinized skin in infect rabbits. Secretory proteins have been reported to play a crucial role in immunoregulation of the host, as well as immune system evasion in parasite infections (Buck et al., 2014; Dogga et al., 2017; Lebrun et al., 2014; Lee, 2009; Mizoguchi, 2006). Parasites can directly secrete proteins into host tissues and fluids, and secretory proteins are known to be important allergens that induce inflammatory responses in the hosts of some parasites (including mites) (Bennuru et al., 2009; Ebner et al., 2014; Fitzsimmons et al., 2014; Hewitson et al., 2009; Levy, 2004). Allergen genes have been identified in the genome of *S. scabiei*, (Rider et al., 2015), and allergen unigenes have been predicted in the transcriptome of *S. scabiei* var *canis*, (Li et al., 2016). In our previous study, unigenes of *S. scabiei* were compared with dust mite allergens, and some homologous allergen unigenes were found (He et al., 2017a). TIMs from *S. scabiei* var *cuniculi* share high homology with the group 25 allergen from the house dust mite. IgE ELISA and IgE dot blotting could not be performed due to the lack of an effective secondary anti-rabbit IgE antibody. Therefore, we preliminarily investigated the allergenic activity of rSs-TIM by intradermal skin tests. The results showed that injection of rSs-TIM into rabbits induced a flare, wheal and erythema

reaction, which are common symptoms of allergic skin responses, suggesting rSs-TIM induced specific and immediate hypersensitivity responses. Moreover, immunolocalization provided evidence of protein distribution and mite invasion in the skin. Together, these results suggest that Ss-TIM may contribute to host invasion by inducing an allergic response in the host, but this response is not suitable for the diagnosis of *S. scabiei*.

Ethics approval

All animals in this study were handled according to the National Standards for Laboratory Animals in China (executed on 05/1/2002) and the animal protection law of the People's Republic of China (released on 09/18/2009). The Animal Ethics Committee of Sichuan Agricultural University, China (Approval No. 2013-028) reviewed and approved this study. Experiments were strictly carried out according to all relevant guidelines and regulations.

Conflicts of interest

The authors have no conflicts of interest to declare.

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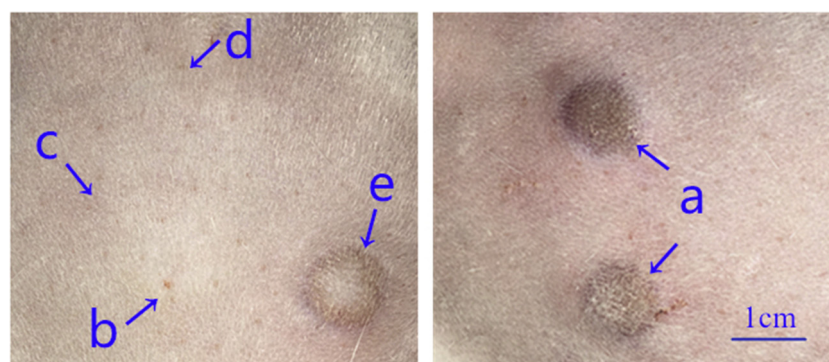


Fig. 7. Intradermal skin tests following injection of rSs-TIM into rabbits. a, purified rSs-TIM (400 µg in 0.1 mL PBS); b, purified pET-32a(+) empty expression vector (400 µg in 0.1 mL PBS); c, 0.1 mL PBS; d, 0.1 mL 0.9% physiological saline; e, 0.1 mL 4 mg/mL histamine.

Authors' contributions

Ran He and Guangyou Yang designed and conceived this study. Ran He performed all the experiments, analyzed the data, and wrote the manuscript. Cheng Guo helped with figure preparation. Haojie Zhang and Nengxing Shen helped with sample collection. Xuerong Peng, Yue Xie, Xiaobin Gu and Weimin Lai help with study design. All of the authors read and approved the final manuscript.

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