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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 suffers (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 (Cowin 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′-CGCGGATCCGTTCCAAGCATTTACCTTTCT-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 *BamH*I 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 Ni⁻NTA His-tag affinity kit (Bio-Rad, USA) according 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*, *Taneia 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 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 ×100) / true positive, and the percentage analytical specificity was calculated as (iELISA negative × 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