A diagnostic test for scabies: IgE specificity for a recombinant allergen of *Sarcoptes scabiei*☆☆☆

Rama Jayaraj a,b, Belinda Hales c, Linda Viberg a, Susan Pizzuto a, Deborah Holt a, Jennifer M. Rolland d, Robyn E. O’Hehir d, Bart J. Currie a,c, Shelley F. Walton a,f,⁎

a Menzies School of Health Research, Darwin, Northern Territory 0810, Australia
b School of Environmental and Life Science, Charles Darwin University, Darwin, Northern Territory 0909, Australia
c Telethon Institute for Child Health Research and Centre for Child Health Research, University of Western Australia, Perth, Western Australia 6872, Australia
d Department of Immunology, Monash University, Melbourne, Victoria 3800, Australia
e Northern Territory Clinical School, Flinders University and Royal Darwin Hospital, Darwin, Northern Territory 0810, Australia
f School of Health and Sport Sciences, University of the Sunshine Coast, Sippy Downs, Queensland 4558, Australia

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Abstract

Scabies infestations are difficult to diagnose clinically and current serologic tests have less than 50% accuracy. To develop more reliable diagnosis of scabies, specific IgE antibodies to a major scabies antigen recombinant Sar s 14.3 (rSar s 14.3) were measured in 140 plasma samples from scabies-infested and control subject groups using dissociation-enhanced lanthanide fluorescent immunoassays (DELFIA). Levels of rSar s 14.3-specific IgE were quantified, and cross-reactivity with its house dust mite homologue, Der p 14, was assessed. The rSar s 14.3 DELFIA showed excellent diagnostic capability, with 100% sensitivity and 93.75% specificity for distinguishing subjects with current scabies infestation from control, uninfested subjects. Recombinant Der p 14 preparation was ineffective at inhibiting IgE binding to rSar s 14.3. This study shows that quantification of levels of IgE antibody to rSar s 14.3 is a highly sensitive method for diagnosis of scabies infestation in clinical practice.

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1. Introduction

Scabies is an infectious skin disease caused by the “itch mite” *Sarcoptes scabiei*. Risk factors for scabies include overcrowding, poor nutrition, and poor health care (reviewed in Walton et al., 2004). In many remote Australian Aboriginal communities, scabies is endemic, with up to 50% of children and 25% of adults infested (Clucas et al., 2008).

Ordinary scabies is the most common form of the disease in humans and is characterized by a low mite burden. This form of the disease is often difficult to diagnose as it mimics a variety of skin conditions, and often no mites can be observed in skin scrapings (Walton and Currie, 2007). The progression of ordinary scabies to the more severe form of the disease, crusted scabies, is unusual and is frequently associated with immunodeficiency, but can also occur in overtly immunocompetent people (Roberts et al., 2005).

Definitive diagnosis of scabies infection currently requires identification of a mite, mite parts, eggs, or mite fecal pellets in skin scrapings. While this method is highly specific, the diagnostic sensitivity is low due to the low of number of mites present in ordinary scabies (Walton and Currie, 2007). A delay in definitive diagnosis can result in the transmission of mites to personal contacts. Thus rapid diagnosis and...
treatment of cases are important to control the spread of the disease. Enzyme-linked immunosorbent assay (ELISA) kits for serodiagnosis of scabies in animals using mite extracts of *S. scabiei* var. *vulpes* are commercially available (Curtis, 2001; Haas et al., 2005; Hollanders et al., 1997).

Serologic assays using whole mite extracts are laborious and expensive because mites must be cultivated from suitable hosts. Additionally, no known in vitro culture system for *S. scabiei* hosts. Additionally, no known in vitro culture system for *S. scabiei* previously identified for serodiagnosis of scabies in animals using mite extracts of *S. scabiei* using a recombinant *S. scabiei* var. *hominis* protein was shown to have 100% sensitivity and 97% specificity in both chamois and deer (Casais et al., 2007).

A fragment of an *S. scabiei* var. *hominis* homologue of the group 14 allergens of HDMs has been identified by immunoscreening of an expression library (Harumal et al., 2003). The group 14 allergens Der p 14 from *Dermatophagoides pteronyssinus* and Eur m 14 from *Euroglyphus maynei* are apolipoporphins that are likely to be major constituents of lipid bodies and lipid transport particles in the haemolymph (Epton et al., 1999, 2001). Presentation of these molecules in lipid particles may increase the immune response (Epton et al., 1999). Recently, our team demonstrated that ordinary and crusted scabies patients showed specific allergic IgE antibody responses to a number of *S. scabiei* recombinant proteins, including the apolipoprotein (Walton et al., 2010).

The usefulness of a serodiagnostic antigen for human scabies is highly dependent on the extent of cross-reactivity, particularly with its HDM allergenic homologues. Therefore, the primary aim of this study was to determine whether a recombinant fragment of the *S. scabiei* var. *hominis* homologue of the group 14 allergens from HDMs (i.e., rSar s 14) could be used in a sensitive and specific diagnostic assay for scabies infestation in humans. We assessed specific IgE binding to recombinant fragments of Sar s 14 (rSar s 14.3) and Der p 14 (rDer p 14) in plasma from subjects with crusted scabies, ordinary scabies, atopic dermatitis, and allergy to HDMs, as well as in plasma from individuals exposed to scabies mites but currently not infested and from naïve individuals.

2. Methods

2.1. Study population and sample collection

Approval for this study was obtained from the Human Research Ethics Committee of the Northern Territory Department of Health and Families and Menzies School of Health Research (approval 97/21). Plasma was collected from 140 consenting volunteers from 6 groups: subjects with crusted scabies (*n* = 30); subjects with ordinary scabies (*n* = 30); previously exposed but currently noninfested subjects (*n* = 30); subjects naïve to scabies (*n* = 30); naïve subjects with atopic allergy as defined by positive skin prick test to a panel of common aeroallergens (*n* = 10); and naïve subjects with known allergy to HDM based on clinical history and positive specific IgE (*n* = 10). Cases of crusted and ordinary scabies were confirmed by clinical observation and/or observation of mites or mite parts in skin scrapings. Samples were stored at −80 °C until assayed.

2.2. Antigens

rSar s 14.3 (AF462196) corresponding to amino acids 1263 to 1655 of Der p 14 (AAM21333) was directionally cloned into the prokaryotic expression vector pQE-9 expressed and purified as described previously (Walton et al., 2010). The homologous fragment of Der p 14 (amino acids 1310 to 1650) was expressed and purified as described previously (Hales et al., 2004). Protein concentrations were determined using Bradford reagent (Bio-Rad Laboratories, Regents Park, NSW, Australia).

2.3. IgE DELFIA

Antigen (rSar s 14.3 or rDer p 14)-specific IgE was measured by dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA™; Wallac, Turku, Finland) with some modifications to the method described previously (Hales et al., 2004). Antigen (0.5 μg per well) was coated onto microtitre plates, left overnight at 4 °C, then washed 5 times in 50 mmol/L Tris-HCl (pH 7)–0.05% Tween 20. This washing step occurred after each incubation, and all incubations were continuously agitated. The plates were blocked with 0.5% bovine serum albumin (BSA) in 50 mmol/L Tris-HCl–0.05% Tween 20 for 1 h at 37 °C, washed, then incubated with diluted (1 in 10 and 1 in 20) subject plasma for 1 h at 30 °C. The plates were then washed, incubated with biotinylated mu-αHu IgE diluted 1 in 1000 in DELFIA assay buffer for 1 h at 30 °C, and washed again. They were next incubated with europium–streptavidin diluted 1 in 1000 in DELFIA assay buffer for 30 min at 30 °C. Enhancement solution (100 μL; Wallac) was then added, and the plates were incubated at 30 °C for 10 min. The fluorescence was measured with a time-resolved fluorometer (VICTOR™ X Multilabel Plate Reader, PerkinElmer, USA) at 405 nm using previously described methods (Schuurman et al., 1997). The average value for the 2 dilutions of each plasma sample was taken as the specific IgE binding of that sample.

2.4. Quantification of total IgE and IgE specific for HDMs

Total IgE and IgE specific for HDMs were determined for subjects with crusted scabies (*n* = 24), subjects with ordinary scabies (*n* = 22), subjects exposed previously but noninfested