



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

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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 (DELFA). 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 DELFA 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 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

Abbreviations: ELISA, enzyme-linked immunosorbent assay; DELFA, dissociation-enhanced lanthanide fluoroimmunoassay; HDM, house dust mite.

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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* var. *hominis* exists. Another complicating factor when using whole mite extracts for serodiagnosis in humans is that antibody cross-reactivity occurs between scabies mites and HDMs (Arlian and Morgan, 2000). Two studies have previously identified *S. scabiei* expressed sequence tags (Fischer et al., 2003; Ljunggren et al., 2006). As a result, a number of proteins from human scabies mites have been cloned and expressed (Dougall et al., 2005; Harumal et al., 2003; Holt et al., 2003; Ljunggren et al., 2006; Mattsson et al., 2001; Pettersson et al., 2005). Recently, an ELISA 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 apolipoproteins 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 (AAM23133) 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™; Wallace, 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 $\mu\alpha\text{Hu}$ 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 ; Wallace) 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

($n = 14$), and naïve subjects ($n = 4$) using the CAP system (Pharmacia Diagnostics, Uppsala, Sweden) as described previously (Hales et al., 2006). These assays were performed to compare total IgE levels with scabies-specific IgE levels and for evidence of sensitization to HDMs.

2.5. Competitive inhibition ELISA

ELISA plates were coated with rSar s 14.3 (0.1 µg per well) at 4 °C overnight, washed with 50 mmol/L Tris-HCl (pH 7)–0.05% Tween 20, blocked, and washed again. Plasma, from a subject with ordinary scabies displaying high level of antibody response to rSar 14.3, was diluted 1 in 160 in 0.1% BSA in phosphate-buffered saline Tween-20 and 50 µL preincubated with 0–50 µg of rDer p 14 or rSar s 14.3 for 60 min at 37 °C, then added to the wells and incubated overnight at 4 °C. Following incubation with rabbit anti-human IgE polyclonal antibody (1 in 4000, 50 µL per well) (Dako, Camberfield, Australia), and then goat anti-rabbit IgG antibody-horseradish peroxidase conjugate (1 in 4000, 50 µL per well) (Promega, Sydney, Australia), the reaction was developed using *o*-phenylenediamine (in phosphate citrate with sodium perborate buffer) and absorbance values were monitored in a plate reader at 490 nm. The percent inhibition was calculated as $100 - [(OD \text{ value of plasma with inhibitor} / OD \text{ value of plasma without inhibitor}) \times 100]$.

2.6. Statistical analysis

Statistical analyses were performed using Prism v. 5.01 (GraphPad Software, San Diego, CA, USA) software. The association between paired and continuous data was estimated using Wilcoxon signed rank tests. Nonparametric Mann–Whitney *U* tests were used to compare the distribution of 2 unmatched groups. Correlations between different assays were assessed with the Spearman correlation. Comparisons were considered to be significant at *P* values of $< .05$. True-positive, true-negative, false-positive, and false-negative values were calculated using a cut-off point of the mean plus 3 SDs of the naïve group.

The receiver operator characteristic (ROC) curve is a graph of the probability of true positivity (sensitivity) versus the probability of false positivity ($100 - \text{specificity}$). The ROC curves and the area under the curve were calculated using Prism for subjects with a current scabies infestation (crusted scabies or ordinary scabies) against subjects with a previous infestation (exposed noninfested), another allergy (atopic or HDM allergic), or no infestation (naïve).

3. Results

3.1. Quantification of specific IgE binding to rSar s 14.3 and rDer p 14

For rSar s 14.3, a clear distinction between specific IgE binding in the plasma from the crusted scabies, the ordinary scabies, and the non–scabies-infested groups was observed

(Fig. 1). The mean IgE binding to rSar s 14.3 for the crusted scabies group was significantly greater compared to each of the other groups ($P < .001$; Fig. 1). The ordinary scabies group also had a significantly higher mean value than each noninfested group ($P < .0001$; Fig. 1). The exposed but noninfested group mean IgE response to rSar s 14.3 was significantly greater than the mean response for the subjects naïve to scabies group ($P < .0004$; Fig. 1).

Although the ranges of rDer p 14–specific IgE levels were similar in all groups, the mean binding in the crusted scabies group was significantly lower than in the ordinary scabies group ($P < .0196$), the exposed but noninfested group ($P = .005$), and the subjects naïve to scabies group ($P = .0412$; Fig. 1).

3.2. Comparison of rSar s 14.3– and rDer p 14–specific IgE binding in crusted and ordinary scabies groups

The mean specific IgE binding to rSar s 14.3 in the crusted and ordinary scabies groups was significantly higher than the corresponding values for rDer p 14 ($P < .001$). There was no significant correlation between the levels of rSar s 14.3–specific IgE and rDer p 14–specific IgE for the crusted scabies group ($r = -0.27$) or the ordinary scabies group ($r = -0.30$).

Interestingly, there was no significant difference between the mean rSar s 14.3 and rDer p 14 specific IgE levels for the HDM allergic group ($P = .16$).

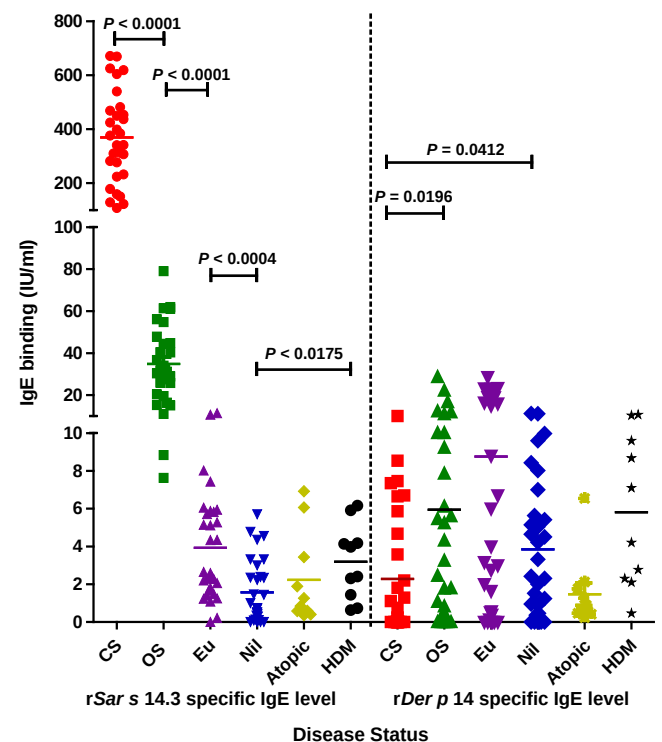


Fig. 1. IgE binding to rSar s 14.3 and rDer p 14 in subjects with crusted scabies (CS), ordinary scabies (OS), exposed but uninfested (EU), naïve to scabies (Nil), atopic, and HDM allergy (HDM). Mean values for each group = bar.

Table 1
Total IgE and IgE specific for HDMs using the CAP system

Disease status	IgE levels (kU/L)			
	Total IgE		HDM specific IgE	
	Median	Range	Median	Range
Crusted scabies	5000	1227–5000	13.65	3.57–60.40
Ordinary scabies	5000	281–5000	13.60	0.35–39.50
Exposed, noninfested	1620	43–5000	2.79	0.35–26.00
Naïve to scabies	47	17–1592	7.2	0.35–16.30

3.3. Comparison of total IgE, HDM-specific IgE, and rSar s 14.3-specific IgE levels

There was no significant correlation between the levels of rSar s 14.3-specific IgE and total IgE for the crusted scabies group ($r = 0.08$) or the ordinary scabies group ($r = 0.09$) (Table 1). Similarly, there was no significant correlation between the levels of rSar s 14.3-specific IgE and the HDM extract-specific IgE for either of these subject groups ($r = 0.16$ and $r = 0.05$, respectively). The crusted scabies and ordinary scabies groups both showed significantly higher levels of total IgE than both the exposed noninfested group ($P < .001$ and $P = .03$, respectively) and the subjects naïve to scabies group ($P < .001$ and $P < .003$, respectively).

3.4. Competitive inhibition study

The antibodies to rSar s 14.3 exhibited very low cross-reactivity to rDer p 14. Preincubation of plasma from a subject with ordinary scabies with rSar s 14.3 resulted in inhibition of subsequent binding to rSar s 14.3 by up to 85%, while preincubation with rDer p 14 did not result in inhibition of subsequent binding to rSar s 14.3 (Fig. 2).

3.5. Diagnostic efficiency of the rSar s 14.3 DELFIA

The rSar s 14.3-specific IgE assay was analyzed for its ability to distinguish a current scabies infestation (crusted scabies or ordinary scabies) from a previous infestation (exposed noninfested) or scabies-naïve. The positive cut-off

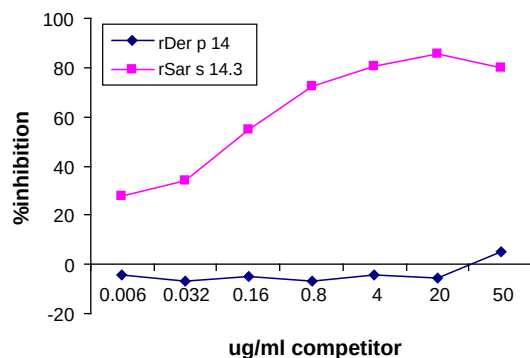


Fig. 2. Competitive inhibition ELISA of rSar s 14.3 binding to rDer p 14 and rSar s 14.3 in plasma from a subject with ordinary scabies.

value for the assay was calculated at 6.6 IU/mL. The diagnostic efficiency of the rSar s 14.3 DELFIA for detecting active scabies infestation was extremely high. The assay had 100% sensitivity, 93.7% specificity, a positive likelihood ratio of 16.0, and a negative likelihood ratio of 0.01. The overall diagnostic accuracy was 96.4% and the area under the ROC curve was 0.999.

4. Discussion

Purified recombinant allergen is becoming increasingly used for diagnostic and therapeutic purposes as a substitute for whole mite extracts because the allergenic components of allergen extracts are often difficult to standardize (Valenta et al., 1999). The DELFIA is an excellent methodology for specific IgE detection in plasma, with its high accuracy and sensitivity, ease of use for multiple samples, and is often used for diagnosis of allergic diseases such as HDM allergy (Hales et al., 2004, 2006; Schuurman et al., 1997). In our previous studies, plasma from subjects with scabies showed a highly increased specific IgE response to a number of recombinant proteins from scabies mites (Dougall et al., 2005; Walton et al., 2010). However, quantification of specific IgE for the abundant antigen Sar s 14.3 indicates the antigen is an excellent target for the standardization of serodiagnosis of scabies. The significant difference in total IgE ($P < .001$) observed between the crusted scabies group and the subjects naïve to scabies suggests a polyclonal response to scabies infestation. This remains to be further investigated.

Our DELFIA with specific IgE antibody quantification for the recombinant antigen Sar s 14.3 showed high sensitivity and specificity with high diagnostic accuracy. Studies measuring IgG antibody cross-reactivity using the var. *vulpes* extract ELISA revealed a sensitivity of only 48% for human scabies patients (Haas et al., 2005), compared with up to 80% for porcine scabies (Hollanders et al., 1997) and more than 84% for canine scabies (Curtis, 2001).

Studies investigating 4 different populations in Europe (Austria: $n = 56$; France: $n = 55$; Italy: $n = 67$; Sweden: $n = 65$) reported that Der p 14 showed weak IgE antibody reactivity to plasma from subjects with HDM allergy compared with other major allergens of HDM (Weghofer et al., 2008). Thus, although Der p 14 is not considered a predominant serodiagnostic antigen for the diagnosis of HDM allergy, its scabies mite homologue, rSar s 14.3, shows high IgE binding for scabies-infested patients in our tests. The advantages of quantification of IgE binding with DELFIA in major peanut allergy and HDM allergy have been reported elsewhere (Hales et al., 2004, 2006). Our results validate the further use of this assay for clinical testing of IgE levels in scabies.

The degree of cross-reactivity between the rSar s 14.3 and Der p 14 proteins were further analysed in a competitive inhibition assay. Our results clearly demonstrate that there is

no cross-reactivity between rSar s 14.3 and HDMs and show that scabies allergy to rSar s 14 is not due to a cross-reactivity with HDM allergy.

The performance of diagnostic tests is generally gauged by measuring sensitivity and specificity (Rosner et al., 1990). ROC plots provide detailed information about the ability to discriminate between positive and negative values, which is also useful in comparing different methods or assays (Soderstrom et al., 2003). An assay with ideal discrimination has an area under the ROC curve of 1 (Zweig and Campbell, 1993). Thus, our assay, with an area under the ROC curve of 0.999, is highly discriminatory for scabies infestation.

In conclusion, our results demonstrate sensitive detection of IgE reactivity with genus-specific scabies mite epitopes and differential diagnosis of scabies mite allergy from allergy to HDM. The rSar s 14.3-specific IgE DELFIA has high diagnostic efficiency, with accuracy superior to other diagnostic methods currently available for scabies. Due to the high sensitivity, specificity, accuracy, predictive values, and area under the ROC curve, the developed DELFIA represents a marked improvement for the clinical diagnosis of scabies and helps direct future development of a specific diagnostic tool for scabies.

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