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Cross-Antigenicity Between the Scabies Mite, *Sarcoptes scabiei*, and the House Dust Mite, *Dermatophagoides pteronyssinus*

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This study demonstrated that antigens of the parasitic mite *Sarcoptes scabiei* (SS) cross-react with antigens of the house dust mite *Dermatophagoides pteronyssinus* (DP). Crossed immunoelectrophoresis (CIE) reaction of SS extract with rabbit anti-DP serum resulted in multiple immunoprecipitates. Reciprocal CIE reactions gave similar results. Immunoprecipitates from both reactions bound IgE in the sera of dust-mite-sensitive patients who had no history of scabies. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis resolved protein/peptide bands of both SS and DP also bound serum IgE from dust-mite-allergic patients following immunoblotting. Non-allergic control sera gave no IgE binding to

either SS or DP antigens. These results indicate that patients with atopy to dust mites exhibit circulating antibodies built to DP but that recognize determinants on SS antigens. It is highly probable that scabietic patients build antibodies to SS antigens that also recognize DP antigens. These results raise questions concerning the reported isotypic antibody responses to SS because the sensitivity of scabietic patients to house dust mites has not been previously evaluated. This cross-reactivity may play an important role in the susceptibility to scabies and its clinical manifestations. *J Invest Dermatol* 96:349-354, 1991

The scabies mite, *Sarcoptes scabiei* (SS), is a common parasite that burrows in the skins of humans and other mammals. The house dust mites, *Dermatophagoides farinae* (DF) and *D. pteronyssinus* (DP), are cosmopolitan inhabitants of human dwellings where they feed on shed skin scales. These mites are sources of potent allergens that stimulate allergic reactions in many dust-sensitive individuals. They occur in most homes in humid geographical areas. Indirect evidence raised the possibility of cross-reactivity between scabies mites and the allergy causing house dust mites. This indirect evidence was that serum IgE levels directed at DP in scabietic patients declined following treatment for scabies [1], scabies patients had positive RAST (radioallergosorbent test) to DP [2], and individuals with skin sensitivity to DP but with no history of previous scabies infestation gave positive skin tests to SS [3]. Falk and Bolle [3] further suggested that atopy may predispose to scabies and even enhance the clinical manifestations of the disease.

An antigenic study revealed that extract made from SS from dogs (var. *canis*) contained nine antigens [4]. By directly reacting SS var. *canis* antigens (extract) with rabbit antiserum built to DF or directly reacting DF extract with sera obtained from SS var. *canis* infested rabbits using crossed immunoelectrophoresis, we directly demonstrated that six of the nine SS antigens were cross-reactive with antigens of DF. Both these cross-reactive SS antigens precipitated by CIE and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) resolved protein/peptide fractions of the SS var. *canis* extract bound IgE when incubated in the sera of *D. farinae* sensitive patients without a history of scabies infestation [5]. Similarly, Falk et al [6] demonstrated four cross-reacting antigens in SS var. *hominis* extract using a CIE reaction with rabbit anti-DF serum. These findings were significant in that they directly established 1) a high level of cross-reactivity between the two mite species, 2) the cross-reactivity can involve IgE type antibodies, and 3) DF-sensitive patients with no history of scabies exhibit circulating antibodies built to DF, but that recognize determinants on SS antigens. Many dust-mite-sensitive patients are skin-test positive to both DF and DP. DF and DP demonstrate cross reactivity but each is also the source of species-specific antigens and allergens. Because both DF and DP are common in homes and are important sources of allergens, the purpose of this study was to directly determine if DP also cross-reacts with SS.

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Abbreviations:

BSA: bovine serum albumin
CIE: crossed immunoelectrophoresis
CRIE: crossed radioimmunoelectrophoresis
DF: *Dermatophagoides farinae*
DP: *Dermatophagoides pteronyssinus*
PAGE: polyacrylamide gel electrophoresis
RAST: radioallergosorbent test
SDS: sodium dodecyl sulfate
SS: *Sarcoptes scabiei*

MATERIALS AND METHODS

Mite Source and Extract Preparation *Dermatophagoides pteronyssinus* (DP) were cultured and mite extracts prepared as previously described [7,8]. Culture medium-free active mites of all life stages were collected by aspiration from culture container rims, killed by freezing, then desiccated under vacuum over Drierite (W.A. Hammond Drierite Co., Xenia, OH). DP fecal material was harvested by sieving (106 μ m) dried, spent cultures in which the food supply had been exhausted. For extract preparation, dried DP material was

Table I. Antibody Response of Sera from *Sarcoptes scabiei*-Infested Rabbits to Antigens in Extract Prepared from DP Body*

Scabietic Rabbit Serum	DP Body Antigen Number						
	Anode						Cathode
	1	2	3	4	5	6	7
A		*	*	*	*	*	*
B		*	*		*	*	
C		*	*	*	*	*	*
D	*		*		*	*	
E		*	*	*	*		
F		*	*		*		
G	*			*	*		*
H			*		*		
I	*		*		*		
J		*			*	*	
K		*	*		*	*	*
L	*		*	*	*	*	*
M		*	*	*	*	*	*
N		*	*	*	*	*	*

* Asterisks denote an antigen-antibody precipitate formed when the scabietic rabbit serum was reacted with DP body extract by CIE.

ground, defatted in anhydrous ethyl ether, placed in the appropriate volume of distilled water to provide a 1:10 wt/vol dilution, and extracted at least 48 h at 4°C. Extract preparations were cold centrifuged and the supernatant filter sterilized (0.20 μ m Acrodisc), and then the extract was stored at 4°C until further use.

Sarcoptes scabiei var. *canis* were grown on New Zealand white rabbits, *Oryctolagus cuniculus*. Mites were collected and extracts prepared as previously described [4,5]. Heavily infested crusts were removed from the rabbits' skin surface and placed in a petri dish so that they encircled a beam of light from a microscope illuminator that was directed through the dish. Mites of all life stages that crawled from the crusts and assembled under the light beam were collected by aspiration every 1–3 h, frozen, and dried over Drierite under vacuum. For extract preparation, dried SS bodies were ground with a micromortar and pestle, defatted in anhydrous ethyl ether, placed in the appropriate volume of distilled water to provide a 1:20 wt/vol concentration, and then extracted for at least 48 h at 4°C. Extracts were cold centrifuged and the supernatant drawn off for use in immunoassays.

Mite Antisera Antisera to DP body and DP feces extracts were raised by subcutaneous immunization of New Zealand white rabbits exactly as described previously [7,8]. The rabbits received initial injections of 1 mg DP protein emulsified in complete Freund's adjuvant and two 0.5 mg doses DP protein in Freund's incomplete adjuvant at two-week intervals. Two weeks following the last booster, blood was drawn from the central ear arteries and the sera separated for immunoassays. SS antisera were obtained from 14 New Zealand white rabbits infested with SS. The rabbits were bled, the sera decanted, and the antibody profiles analyzed by crossed immunoelectrophoresis (CIE). The sera of the four rabbits with the highest antibody titers to SS were pooled, concentrated 2.5X, and used for the crossed radioimmuno-electrophoresis (CIE) analyses.

CIE To determine the cross-reactivity of SS and DP, heterologous CIE was performed using procedures identical to those reported in earlier studies [4,5,7–9]. For these analyses, SS extract was reacted with DP antisera and DP extracts were reacted with SS antisera. In the first dimension, 14–20 μ l of SS or DP extracts were horizontally electrophoresed for 20 or 30 min, respectively, at 200 V. This was followed by second-dimension electrophoresis in

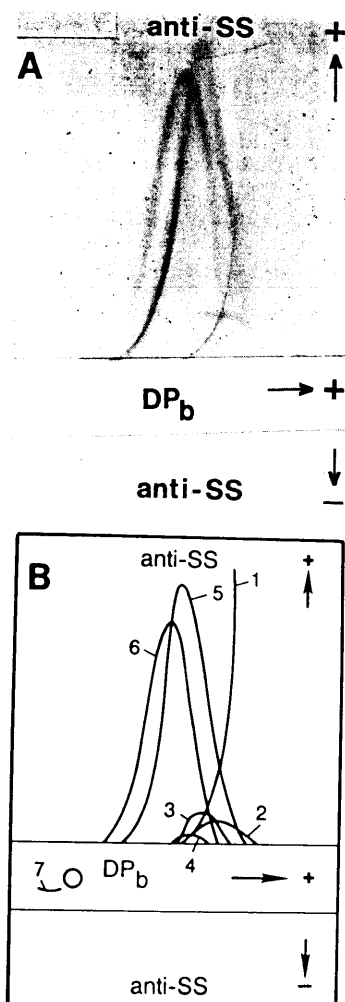


Figure 1. DP body (DP_b) extract reacted with sera from scabies infested rabbits (anti-SS). A: representative CIE antigen profile. B: composite schematic.

which the extracts were perpendicularly electrophoresed into an agarose gel containing the heterologous antiserum. When using DP extract, anodic and cathodic second-dimension gels contained 500 μ l and 100 μ l of anti-SS serum, respectively. When SS extracts were reacted with anti-DP, the anodic and cathodic gels contained 200 and 10 μ l of anti-DP that had been concentrated 5X. SS, DP body, and DP feces extracts reacted with normal rabbit serum served as negative controls. Second-dimension electrophoresis was performed with 40 V for 15–20 h. The gels were then washed, pressed, dried, stained with Coomassie Brilliant Blue R-250 (Sigma Chemical, St. Louis), and destained.

CRIE CRIE was performed to determine if patient IgE built to DP would bind to SS and DP antigenic determinants. CIE gels of DP reacted with anti-SS that were used for CRIE contained 300 and 20 μ l of pooled anti-SS serum (concentrated 2.5X) in the anodic and cathodic gels, respectively. The reciprocal gels (SS reacted with anti-DP) contained 350 μ l of anti-DP in the anodic gel. CRIE using gels on which DP was reacted with DP served as positive controls. The homologous CRIE gels contained 400 and 60 μ l of antiserum in the anodic and cathodic gels, respectively. These unstained CIE gels were incubated overnight in sera from DP-sensitive patients and RAST A buffer [9]. They were then washed 3 times with RAST

Table II. Antibody Response of Sera from *Sarcoptes scabiei*-Infested Rabbits to Antigens in Extract Prepared from DP Feces*

Scabietic Rabbit Serum	DP Feces Antigen Number				
	Anode				Cathode
	1	2	3	4	5
A	*	*			*
B	*	*		*	*
C		*			*
D	*	*	*		*
E		*		*	*
F		*	*	*	*
G		*		*	*
H	*	*		*	*
I	*	*		*	*
J	*	*	*	*	*
K	*	*	*	*	*
L	*	*	*	*	*
M	*	*	*	*	*
N	*	*	*	*	*

* Asterisks denote an antigen-antibody precipitate formed when the scabietic rabbit serum was reacted with DP feces extract by CIE.

B buffer, incubated with ^{125}I anti-human IgE and RAST C buffer, washed, pressed, and air dried. Autoradiography was performed for 1-26 d.

SDS-PAGE, Blot Transfer, and Autoradiography Proteins in the SS and DP mite extracts were separated by standard SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) using 12% polyacrylamide as described previously [5]. Twenty-five to thirty microliters of mite extracts were resolved for each electrophoresis run. BSA (bovine serum albumin), ovalbumin, trypsinogen, and lysozyme were run as molecular weight markers. Reference gels were stained with silver nitrate to visualize the protein-peptide bands. All gels were stained following blot transfer to verify complete transfer.

Separated proteins were transferred at 280 mA and constant current for 2 h to nitrocellulose membranes using a Bio-Rad Transblot cell system (Bio-Rad Laboratories, Rockville Centre, N.Y.). Nitrocellulose strips containing proteins were treated identically to the CRIE gels, except that each was washed 3 more times with Tris-saline solution after the last RAST B buffer wash. The strips were incubated in patients' sera overnight, washed, and incubated in ^{125}I -labeled anti-human IgE overnight, and then autoradiography was performed.

Human Sera CRIE gels and transblot strips were incubated in sera from 15 DP-sensitive patients. These individuals were selected on the basis of persistent allergic symptomatic histories during the heating season, positive prick or intracutaneous skin tests (≥ 3 mm wheal and flare) to a commercial DP extract and without prior history of SS infestation. Sera from 10 individuals who were skin test negative to DP and had no history of scabies served as negative controls.

RESULTS

Cross-Reactivity of DP Body/Feces and SS Extracts Heterologous CIE reactions of DP body and DP feces extracts with antisera from 14 individual scabies-infested rabbits showed that the rabbits contained serum antibodies directed at DP body and DP feces antigens. Seven different antigen-antibody precipitin lines (six anodal and one cathodal) were apparent when DP body extract was

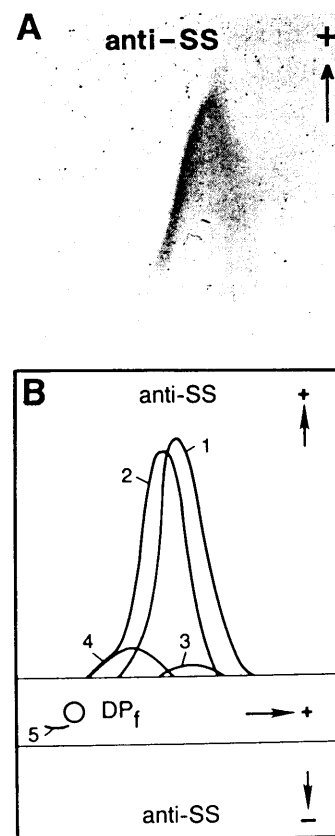


Figure 2. DP feces (DP_f) extract reacted with sera from scabies-infested rabbits (anti-SS). A: representative CIE antigen profile. B: composite schematic.

reacted with individual sera from SS-infested rabbits (Table I, Fig 1). Varying numbers of cross-reacting DP body antigens were recognized by individual rabbit's serum but all rabbit sera contained antibodies that recognized at least two cross-reacting DP body antigens. Different rabbits' sera gave stronger more clearly defined antigen-antibody precipitates for different antigens. Thirty-six percent of the scabietic rabbits recognized six DP body antigens, whereas 7% recognized only two antigens. All rabbits recognized cross-reacting antigen number 5, whereas 86% of the rabbits had antibodies directed at cross-reacting antigen number 3. Reciprocal CIE reactions of SS extract and DP body antiserum detected multiple cross-reacting antigens also. Sera from control rabbits exhibited no antibodies to DP body or SS antigens when reacted with either extract.

CIE reactions of DP feces extract with individual sera from the same 14 SS-infested rabbits exhibited up to five different precipitin lines (Table II, Fig 2). The number of cross-reacting DP feces antigens recognized by an individual serum varied from one to four. Half of the rabbits recognized four cross-reacting feces antigens. Eighty-six percent of the rabbits contained antibodies directed at cross-reacting antigens numbers 2 and 5. Fifty-seven and fifty percent had antibodies against antigens numbers 1 and 4, respectively. Reciprocal heterologous CIE crosses also demonstrated antigen-antibody reactions.

IgE Antibody Binding to Cross-Reactive DP and SS Antigens Heterologous CIE gels containing the antigen-antibody precipitates that had formed from the cross-reaction of DP body extract and anti-SS serum were individually incubated with sera of 15 DP-sensitive patients who had no history of SS infestation. Autoradiograms of these plates indicate that five different DP body antigens

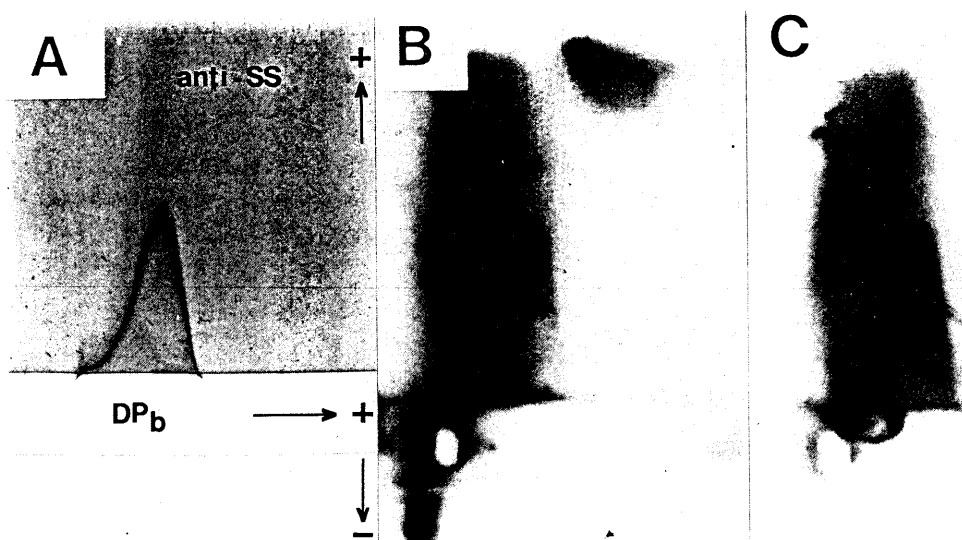


Figure 3. A: CIE antigen profile of DP body extract reacted with pooled rabbit anti-SS. B, C: IgE binding patterns (autoradiograms) for two house dust mite-sensitive patients with no history of scabies.

that cross-reacted with SS, bound mite-specific IgE (Fig 3). Fifteen and 10 DP-sensitive patients exhibited IgE binding to cross-reacting DP body antigens numbers 5 and 6, respectively. The number of cross-reacting antigens that bound IgE and exhibited radiostaining for an individual serum ranged from two to four. Each serum showed varying levels of IgE binding to different precipitin lines as indicated by the varied intensity of radiostaining. Autoradiograms for the reciprocal cross in which SS extract was reacted with DP body antiserum also showed that IgE built to DP antigens bound to the SS antigens (Fig 4). Likewise, nine of the 15 dust mite-sensitive patients without a history of scabies exhibited IgE binding to immunoprecipitates resulting from the homologous CIE reaction of SS extract with rabbit anti-SS sera (Fig 5). Control sera from 10 individuals skin test negative to DP and with no history of SS infestation showed no IgE binding (blank autoradiograms) for the three reactions.

More than 30 protein/peptide bands were resolved by SDS-PAGE fractionation of DP body and SS extracts (Fig 6). These DP and SS protein/peptide tracks were blot transferred to nitrocellulose

membranes and then individually incubated with sera of the 15 DP-sensitive patients followed by ¹²⁵I-labeled anti-human IgE. IgE in the sera from dust mite-sensitive patients bound to both DP and SS bands (antigenic determinants) (Table III, Fig 6). Eight different protein/peptide bands from the SS extract bound IgE to varying degrees. Sera from 11 of the 15 patients showed IgE binding although four exhibited only weak binding. All 11 positive patients showed IgE binding to at least two SS bands with five showing binding to six different bands. Sera from all patients exhibited strong IgE binding to at least one DP band with 10 showing varied levels of binding to three or more bands. Twelve different DP bands bound IgE among the various patients. By comparison, the level of IgE binding was greater for DP than SS as measured by duration of autoradiography and intensity of radiostaining. The 10 control sera (skin test negative to DP) exhibited no IgE binding to either SS or DP protein/peptide bands.

DISCUSSION

Heterologous CIE results of this study demonstrated that antigens of *S. scabiei* (SS) cross-react with antigens of *D. pteronyssinus* (DP). IgE in the sera of dust mite-sensitive individuals that had no history of scabies bound to both SS and DP antigenic determinants on immunoprecipitates that resulted from heterologous CIE reactions. When SS extract was used the IgE bound directly to determinants on the SS antigens in the SS/anti-DP immunoprecipitates. When DP extracts were used IgE bound to DP determinants on antigens that had also reacted with antibodies built to SS. In addition, serum IgE from these allergic patients bound directly to SDS-PAGE resolved DP and SS protein/peptide bands. Non-allergic controls gave no IgE binding to either SS or DP antigens precipitated in heterologous and homologous CIE reactions or when resolved by SDS-PAGE. These results indicate that DP-sensitive patients with no history of scabies exhibit circulating antibodies built to DP but that recognize determinants on SS antigens. It is also highly probable that scabetic patients exhibit circulating antibodies built to SS antigens that recognize DP antigens.

Few controlled studies have directly investigated and characterized the isotypic humoral response to scabies antigens, identified predisposing host properties that would explain the spread of scabies and fluctuations in its incidence, or classified how the humoral response to scabies antigens relates to type I, II, III, and IV reactions. This and previous studies indicating a high level of cross-reactivity between scabies mites and the allergy causing house dust mites [5,6]

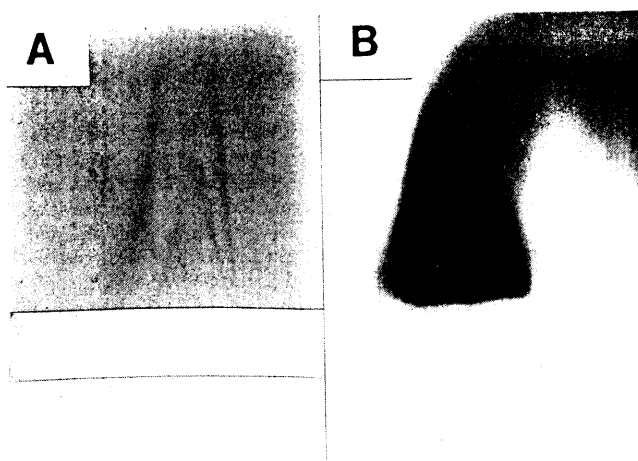


Figure 4. A: CIE antigen profile of SS extract reacted with rabbit anti-DP body serum. B: representative IgE binding pattern (autoradiogram) using a house dust mite-sensitive patient with no history of scabies.

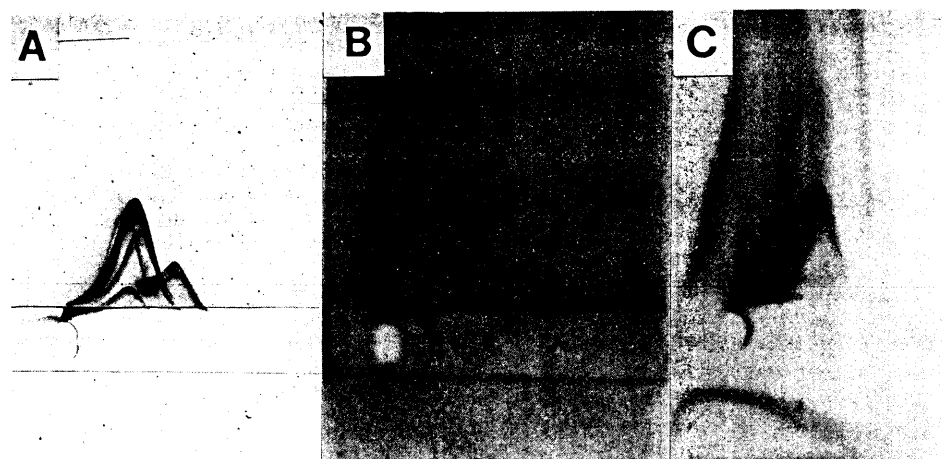


Figure 5. A: CIE antigen profile of SS extract reacted with pooled scabies infested rabbit sera. B, C: representative IgE binding patterns (autoradiograms) using two house dust mite-sensitive patients with no history of scabies.

pose important questions concerning the reported circulating antibody response to scabies and future studies of the humoral response. Because no previous study that described the antibody profiles of scabietic patients considered the patients' sensitivity to house dust mites, it is now unclear whether the few reports of circulating IgE, IgA, IgM, and IgG levels for scabies patients actually reflect a response to scabies antigens [1-3,10-17]. Studies utilizing both dust mite-allergic and non-allergic scabietic patients are now needed

Table III. Number of SDS-PAGE-Resolved Protein/Peptide Bands of DP Body or SS Extracts that Bound IgE After Incubation in Individual Sera from Patients Skin Test Positive to DP

Patient Serum	Number of Protein-Peptide Bands that Exhibited IgE Binding ^a					
	DP body			SS		
	S	M	W	S	M	W
-A	1	1	4	0	0	2
LA	1	0	0	0	0	0
EC	7	4	1	2	3	0
JD	1	0	0	0	0	1
KE	10	2 ^b	?	1	3	0
SK	5	1	?	3	3	0
LL	1	1	0	0	0	0
-M	3	1	0	1	0	3
KM	9	0	0	1	0	3
PM	1	0	0	0	0	2
YO	2	0	0	1	0	0
BR	5	1	?	0	0	0
CT	1	1	0	0	0	0
JT	4	4	3	0	0	3
KW	5	1	?	1	3	0
-A ^c	0	0	0	0	0	0
KC ^c	0	0	0	0	0	0
JD ^c	0	0	0	0	0	0
ND ^c	0	0	0	0	0	0
MG ^c	0	0	0	0	0	0
BG ^c	0	0	0	0	0	0
LL ^c	0	0	0	0	0	0
MP ^c	0	0	0	0	0	0
BS ^c	0	0	0	0	0	0
RW ^c	0	0	0	0	0	0

^a S, strong; M, moderate; W, weak IgE binding after 8 d autoradiography.
^b ?, number of bands could not be determined due to heavy staining.
^c Control, skin test negative to DP and with no history of *S. scabiei* infestations.

both to characterize the antibody response to scabies and to clarify its relationship to house dust mite allergy.

Clearly, an immunologic relationship between scabies and house dust mites exists. Although the clinical and diagnostic implications of this remain to be determined for atopic dust mite and non-allergic individuals, this high level of cross-reactivity may play a significant role in human susceptibility to scabies or in the severity of symptoms. It has been suggested that individuals sensitive to dust mites are more likely to develop scabies than non-allergic individuals [2]. This remains to be documented and, if true, the mechanism explained. More severe skin rash in atopic scabietic patients by comparison to non-allergic scabietic individuals has been reported but needs further investigation [2]. This may be an expression of the cross-reactivity between the two mites. Clinical entities of post-scabietic pruritus of primary scabies that may last weeks to months in some individuals may better be explained by this phenomenon. Likewise, pruritus of primary infestations that develop within a few days to 2 weeks in some cases [18-20] rather than the usual 4-8 weeks [21] may also be due to the individuals being sensitive to house dust mite antigens.

Many studies report elevated IgE values in highly varied percentages (1 to 65%) of the examined scabietic patients and suggest an allergic response to scabies antigens [1-3,10,11,15-17]. A major difficulty in interpreting the varied results of these studies is that most did not directly use SS antigens, because little or no scabies antigen was available for specific determinations. Therefore, it is not certain if these abnormal levels of immunoglobulins or any quantitative relationship between isotypes were antigen-specific or non-specific, associated with accompanying cutaneous bacterial pyoderma infections that are usual sequelae [12] or, in the case of IgE, the result of atopy. Therefore, direct evidence that SS antigens stimulate IgE production and an allergic response in scabietic patients still requires further investigation. However, the fact that IgE-type antibodies in scabies-free dust mite allergic patients will bind to epitopes on SS antigens is indirect evidence that SS can induce IgE production and an allergic response in humans.

This cross-reactivity between SS and DP appears to be one of partial antigen identity. Some antigens of the two mite species have some identical or nearly identical epitopes but molecules of each also have some species-specific epitopes. Two lines of evidence suggest this. First, the immunoprecipitates that formed in the gel of heterologous CIE reactions were less well defined than those formed for homologous reactions. This would suggest low amounts of antibodies or antibodies with low affinities for the strange antigens. Second, using serum from the house dust mite-sensitive patients there was much less IgE binding to SS protein/peptide bands

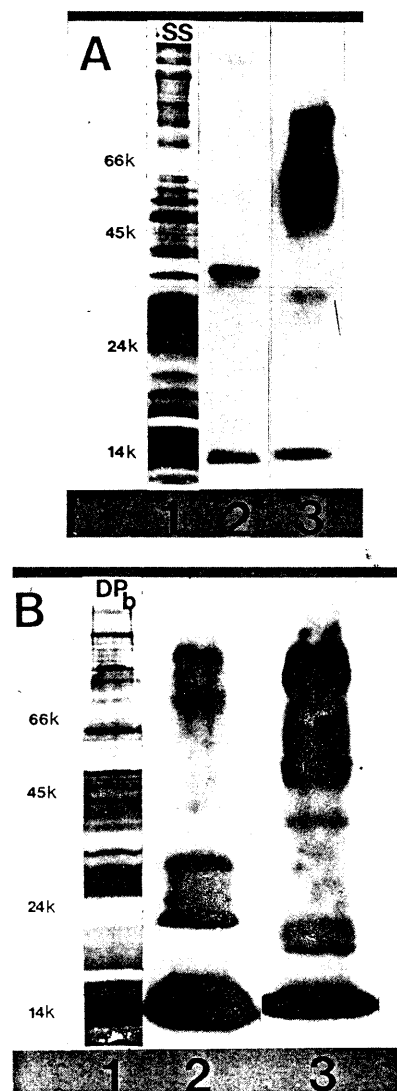


Figure 6. A: SDS-PAGE fractionation profile of (A1) SS extract, (B1) DP body extract. Corresponding numbers in A and B are the same patient. 2,3: representative immunoblots (autoradiograms) showing IgE binding using two dust mite-sensitive patients with no history of scabies.

by comparison with IgE binding to DP bands for each individual sera.

Sarcoptes scabiei var. *canis* was used for these studies because of the difficulty in obtaining large numbers of *S. scabiei* var. *hominis* for extract preparation. Strains of *S. scabiei* from different host species are host specific and normally cannot be transferred between hosts [22,23]. We have successfully transferred var. *canis* from the dog to laboratory rabbits [22,23]. Similar attempts to transfer var. *hominis* to rabbits or other hosts have failed [22,23]. This strain raised on rabbits has been used for recent immunologic studies and as a model for human scabies. The various strains of *S. scabiei* are morphologically alike but the strict host specificity of the strains suggests there are some physiologic differences. However, indirect evidence suggests var. *canis* and var. *hominis* are antigenically similar and may differ by only two antigens [5,6]. Therefore var. *canis* used in this study shares sufficient antigens with var. *hominis* to serve as a good

model for directly demonstrating the immunologic relationship between human scabies and house dust mite atopy.

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