

Circulating IgE in Patients with Ordinary and Crusted Scabies

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J. Med. Entomol. 41(1): 74-77 (2004)

ABSTRACT Serum from seven patients with ordinary scabies and six with crusted scabies were screened by immunoblotting for IgE- and IgG-specific proteins in an extract of the mite, *Sarcoptes scabiei* variety *canis*. Sera from atopic individuals without sensitivity to house dust mites were used as controls. Serum from all of the patients with crusted scabies showed strong IgE binding to 11-21 and IgG binding to 1-7 scabies proteins. In contrast, three of the seven patients with ordinary scabies showed IgE binding to one to six scabies proteins, and their antibody binding was much weaker. Patients with crusted scabies had serum antibody that reacted with larger molecular weight proteins compared with patients with ordinary scabies. The results of this study indicate that patients with crusted scabies showed a pronounced IgE response to scabies mites, whereas patients with ordinary scabies did not.

KEY WORDS *Sarcoptes scabiei*, scabies, antibody, IgE

SCABIES IS AN IMPORTANT disease of humans and many species of mammals worldwide. The mite *Sarcoptes scabiei* burrows in the stratum corneum of the skin epidermis, causing the disease. During both primary and secondary infestations, a severe itching and rash develop. In humans, clinical symptoms of a primary infestation (sensitization) are slow to develop and may not be evident for 4-8 wk (Mellanby 1944). During a secondary infestation in humans, symptoms usually develop in 1-3 d. Dogs and pigs infested with *S. scabiei* (varieties *canis*, *vulpes*, or *suis*) show a similar pattern, with the primary infestation symptoms developing in 2-5 wk, whereas they are evident in 1-2 wk in reinfested dogs and pigs (Davis and Moon 1990, Bornstein and Zakrisson 1993a, b, Arlian et al. 1996, Arlian and Morgan 2000). In dogs, pigs, rabbits, and humans, a primary infestation induces partial or complete immunity to subsequent exposure if the first infestation is cured by treatment (Mellanby 1944, Arlian et al. 1994a, 1996).

Associated with these clinical manifestations during both primary and secondary infestations are inflammatory and immune responses by the host. Scabietic lesions are characterized by pronounced extravasation of neutrophils, Langerhans cells, plasma cells, CD4+ and CD8+ T-lymphocytes, and weaker infiltrates of macrophages, eosinophils, and mast cells

(Hejazi and Mehregan 1975, Sheahan 1975, Ackerman 1977, Falk and Eide 1981, Falk and Matre 1982, Renunala et al. 1984, Head et al. 1990; Arlian et al. 1994b, 1997, Stemmer et al. 1996). The reported composition of the cellular infiltrate varies between host species and between studies of the same host species. Most of the variation in reports from different investigators is likely because of the fact that biopsies were taken at different times during the progressive course of the infestation. It is clear that the cellular infiltrate develops before serum antibody to scabies proteins can be detected during a primary infestation. This has made development of an antibody-based blood diagnostic test difficult.

A humoral immune response does develop during both the primary and secondary infestation. The serum antibody titer develops slowly but intensifies to a plateau as the primary infestation progresses (Davis and Moon 1990, Bornstein and Zakrisson 1993a, b, Arlian et al. 1996, Arlian and Morgan 2000). Animal hosts that do not develop a protective response to a secondary infestation exhibit high antibody titers and weaker cellular infiltrates compared with protected hosts (Arlian et al. 1994a, b, 1996). This suggests that protective immunity is associated with an upregulated Th1 response (Arlian et al. 1995).

The most common form of the disease in humans is "ordinary scabies" and is characterized by a low parasite burden. This form is often difficult to diagnose because it presents with a variety of dermal manifestations, and very few mites, if any, can be located in the skin by taking skin scrapes. "Crusted scabies," also known as Norwegian scabies in humans, is characterized by hyperkeratosis and a high parasite burden. The density of mites may be very high, with reported

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densities of 1,500 mites/cm² of skin under a crust (Arlian et al. 1988), 18,000 mites/g of crust (Dobson 1981), or 4,700 mites/g of shed human skin (Currie et al. 1995). In dogs, pigs, and rabbits, a primary infestation almost always progresses to crusted scabies (scabs). In humans, this condition (Norwegian scabies) is not often seen, and it is not clear what factors prevent or lead to its development. When crusted scabies is observed, it seems to be associated with older patients that may be immunocompromised or in younger patients that are immunosuppressed (e.g., AIDS patients), although this is not always the case. It is not clear if the advanced state (crusting) is because of features of the inflammatory/immune response of the host or if a heavy mite burden develops on the host as a result of lack of grooming, poor hygiene, or a depressed inflammatory/immune response that allows the mites to multiply unchecked and that this heavy mite burden stimulates the hyperkeratosis. Many cells undergoing mitosis are observed in the basal keratinocyte layer of the epidermis under scabietic crusts (Arlian et al. 1994b).

The purpose of this study was to identify differences in the serum antibody profiles of patients with normal (ordinary) and crusted (Norwegian) scabies. To help understand the host's immune response to scabies and to identify relevant antigens for inclusion in a vaccine, we compared serum IgE and IgG antibody profiles to antigens of scabies mites for patients with ordinary and crusted scabies.

Materials and Methods

Study Subjects. Serum was collected from scabietic patients under protocols that were reviewed and approved by the Institutional Review Board of Wright State University. In addition, the Human Research Ethics Committee of the Northern Territory Department of Health and Community Services and the Menzies School of Health Research also reviewed and approved the protocols for collection and use of serum from crusted scabies patients. All scabietic subjects provided informed consent and had an active scabies infestation that was confirmed by the recovery of live *S. scabiei* mites by skin scrapes or from crusts.

Control subjects were atopic individuals who were not allergic to dust mites but that had high circulating IgE levels to other allergens. These sera, the age and sex of the subjects, and pertinent radioallergosorbent test, RAST data were provided to us without personal identifiers by the Laboratory Corporation of America.

Mite Extracts. An aqueous extract of the scabies mite *Sarcoptes scabiei* var. *canis* (SS), was prepared from homogenized mite bodies as previously described (Morgan and Arlian 1994). Total protein concentration in the extract was determined by the method of Bradford (1976).

Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis/Immunoblotting. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were performed to screen the patients' sera against scabies mite proteins and to deter-

Table 1. Number of IgE- and IgG-binding bands detected on immunoblots probed with human serum

Subject	Age	Sex	Number of Ig-binding bands	
			IgE	IgG
Ord 1	68	M	2	0
Ord 2	70	M	0	0
Ord 3	72	M	0	1
Ord 4	45	F	0	0
Ord 5	29	F	0	0
Ord 6	20	M	6	0
Ord 7	60	M	1	1
Cr 1	63	M	14	2
Cr 2	45	M	21	7
Cr 3	32	F	11	4
Cr 4	35	F	17	7
Cr 6	62	F	14	4
Cr 7	29	M	14	1

Serum from patients with ordinary scabies (Ord) was diluted by 1/5, where that from crusted scabies patients (Cr) was diluted by 1/50.

mine the molecular weights of IgE- and IgG-binding proteins as previously described (Morgan et al. 1997). Electrophoresis was carried out on 12% acrylamide mini-gels (Bio-Rad, Hercules, CA) under nonreducing conditions using the method of Laemmli (1970). Proteins were transferred to Pro-Blott polyvinylidene difluoride membrane (Applied Biosystems, Foster City, CA) in 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid and 10% methanol (pH 11) according to the manufacturer's recommendations. Blots were blocked overnight, washed, and probed with serum diluted 1/5 for the controls and ordinary scabies patients and 1/50 for those with crusted scabies. IgE binding was detected using 0.5 μ Ci ¹²⁵I-anti-human-IgE followed by autoradiography for various exposure times. After autoradiography, IgG antibody binding to the blots was detected using peroxidase-labeled goat anti-human-IgG (Fisher, Pittsburgh, PA) at 1:1,000. All blots were developed for 5 min using the 4-chloro-1-naphthol/3,3'-diaminobenzidine/H₂O₂ (CND) chromogenic substrate of Young (1989). Blots and autoradiograms were analyzed, and molecular weights calculated using a Kodak EDAS 120 imaging system (Eastman Kodak Co., Rochester, NY) with 1D analysis software.

Results

Patients with Ordinary Scabies. Few patients that presented with confirmed ordinary scabies (positive skin scrape) had circulating IgE and/or IgG directed at proteins in an extract of SS (Table 1). Three of the seven patients had serum IgE directed at SS proteins, and two of the seven had circulating IgG to SS proteins. Only one of these patients had both IgE and IgG directed at SS antigens. One patient had IgE directed at six different proteins. A second patient had IgE directed at two proteins, and a third had IgE for one protein. Two patients had IgG that recognized one protein each. IgE binding was directed mostly at proteins of <90 kDa (Table 2; Fig. 1). Eight different

Table 2. Immunoblot results showing summary of binding of IgE and IgG from the serum of patients with ordinary ($n = 7$) and crusted ($n = 6$) scabies to proteins of *S. scabiei* var. *canis*

Band no.	MW (kDa)	Number of patients with scabies-specific Ig			
		Ordinary scabies		Crusted scabies	
		IgE	IgG	IgE	IgG
1	470	—	—	3	1
2	440	—	—	3	—
3	425	—	—	3	—
4	400	—	—	2	—
5	380	—	—	4	1
6	257	—	—	2	—
7	246	2	1	6	3
8	220	—	—	6	6
9	204	—	—	6	—
10	158	—	—	6	2
11	123	—	—	6	3
12	103	—	—	6	3
13	89	1	—	6	3
14	69	1	—	5	1
15	52	1	—	5	—
16	36	1	1	4	—
17	34	1	—	2	—
18	30	1	—	5	—
19	27	—	—	3	1
20	23	—	—	2	—
21	21	1	—	5	1
22	13	—	—	3	—
23	10	—	—	2	—

proteins bound IgE, but only two different proteins bound IgG, and both of these also bound IgE.

Patients with Crusted (Norwegian) Scabies. All six patients with crusted scabies had both circulating IgE and IgG that recognized many SS proteins (Table 1). The IgE and IgG binding proteins ranged from 10 to 470 kDa (Table 2; Fig. 1). Individual patients had serum IgE that was directed at 11–21 different proteins (Table 1). Eleven different proteins (21–246 kDa) were recognized by IgE in the serum of five or more of these patients. The proteins that were allergenic for the largest number of patients were in the 52- to 246-kDa range.

All six of the crusted scabies patients also had IgG directed at SS proteins. The number of IgG binding proteins that an individual patient recognized ranged from one to seven. Four of the six patients had serum IgG directed at four or more proteins.

Eleven proteins bound both IgE and IgG. Twelve proteins bound only IgE and not IgG.

Atopic Subjects Not Sensitive to Dust Mites. Atopic subjects that were not sensitized to house dust mites had no serum IgE or IgG directed at scabies (data not shown).

Discussion

Our investigation found that few patients with ordinary scabies had serum IgE and/or IgG directed at scabies proteins. Except for one patient, the number of immunogenic proteins that were recognized was small. In contrast, patients with crusted scabies had circulating IgE and IgG type antibodies to many SS

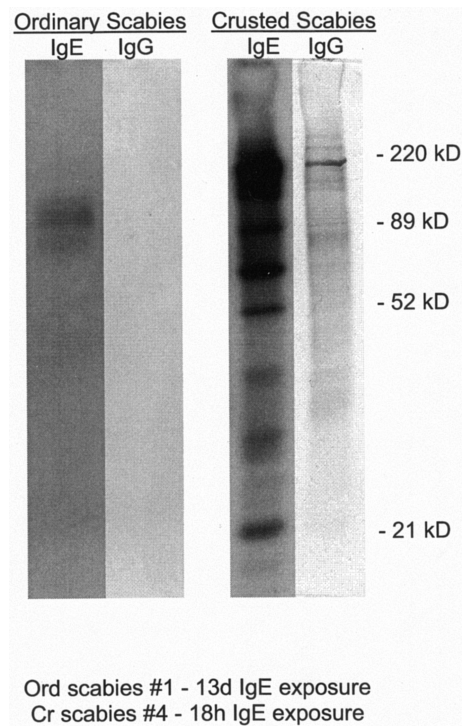


Fig. 1. Representative immunoblots showing serum IgE and IgG binding to scabies proteins separated by SDS-PAGE on 12% gels. Serum was from ordinary-scabies subject 1 diluted 1/5 and crusted-scabies subject 4 diluted 1/50. Molecular weights of selected proteins are shown at right.

proteins compared with the patients with ordinary scabies. Importantly, these antibodies were detected in serum that was probed at 1/50 for crusted scabies patients, whereas serum from subjects with ordinary scabies was analyzed at 1/5. Therefore, patients with ordinary scabies exhibit weak circulating antibody responses to scabies antigens compared with the strong antibody responses presented by patients with crusted scabies.

Significantly, the crusted scabies patients had stronger IgE responses compared with their IgG responses. Crusted scabies patients had serum IgE to large numbers of SS proteins but IgG to many fewer proteins. It seems that chronic infestation with scabies induces a strong IgE (allergic) and a weaker IgG response in infested patients. This suggests that the major response by crusted scabies patients is an allergic reaction. It also raises the following question: do these mites produce substances that downregulate the IgG and IgE immune responses, in particular the balance between the Th1 and Th2 responses? Is the difference between ordinary scabies and crusted scabies in humans related in some way to how mites influence the balance between a Th1 response in protected hosts and the strong Th2 response of unprotected hosts observed in animals in animal studies? This remains to be investigated.

The molecular weights of the immunogenic proteins that scabietic patients recognized ranged from 10 to 470 kDa. We identified 23 different molecules from 10 to 470 kDa that trigger IgE and/or IgG production by scabietic patients. Interestingly, patients with crusted scabies had serum antibody that recognized larger molecular weight proteins compared with the patients with ordinary scabies. The significance of this is not clear.

Acknowledgments

Serum from the atopic control subjects was provided by the Laboratory Corporation of America. The authors thank C. M. Rapp for technical assistance with the experiments. This work was supported by National Institute of Allergy and Infectious Diseases Grant AI-17252, Australian National Health and Medical Research Council Grant 137205, and the Cooperative Research Centre for Aboriginal and Tropical Health.

After this research was concluded but before the final manuscript was completed, one of the authors, S.A.E., passed away suddenly (May 5, 2003). We are grateful for his important contribution to this project, his great enthusiasm for scabies research, and his concern and care for patients with scabies. In recognition of his contribution to this study, his name appears as an author.

References Cited

- Ackerman, A. B. 1977. Histopathology of human scabies, pp. 88–95. In M. Orkin, H. I. Maibach, L. C. Parish, R. M. Schwartzman (eds.), International conference on scabies, 1976. Scabies and pediculosis. Lippincott, Philadelphia, PA.
- Arlan, L. G., and M. S. Morgan. 2000. Serum antibody to *Sarcoptes scabiei* and house dust mite prior to and during infestation with *S. scabiei*. *Vet. Parasitol.* 90: 315–326.
- Arlan, L. G., M. Ahmed, D. L. Vyszynski-Moher, S. A. Estes, and S. Achar. 1988. Energetic relationships of *Sarcoptes scabiei* var. *canis* (Acari: Sarcoptidae) with the laboratory rabbit. *J. Med. Entomol.* 25: 57–63.
- Arlan, L. G., M. S. Morgan, D. L. Vyszynski-Moher, and B. L. Stemmer. 1994a. *Sarcoptes scabiei*: the circulating antibody response and induced immunity to scabies. *J. Exp. Parasitol.* 78: 37–50.
- Arlan, L. G., C. M. Rapp, D. L. Vyszynski-Moher, and M. S. Morgan. 1994b. *Sarcoptes scabiei*: histopathological changes associated with acquisition and expression of host immunity to scabies. *J. Exp. Parasitol.* 78: 51–63.
- Arlan, L. G., C. M. Rapp, and M. S. Morgan. 1995. Resistance and immune response in scabies-infested hosts immunized with *Dermatophagoides* mites. *Am. J. Trop. Med. Hyg.* 52: 539–545.
- Arlan, L. G., M. S. Morgan, C. M. Rapp, and D. L. Vyszynski-Moher. 1996. The development of protective immunity in canine scabies. *Vet. Parasitol.* 62: 133–142.
- Arlan, L. G., C. M. Rapp, B. L. Stemmer, M. S. Morgan, and P. F. Moore. 1997. Characterization of lymphocyte subtypes in scabietic skin lesions of naive and sensitized dogs. *Vet. Parasitol.* 68: 347–358.
- Bornstein, S., and G. Zakrisson. 1993a. Humoral antibody response to experimental *Sarcoptes scabiei* var. *vulpes* infection in the dog. *Vet. Dermatol.* 4: 107–110.
- Bornstein, S., and G. Zakrisson. 1993b. Clinical picture and antibody response in pigs infected by *Sarcoptes scabiei* var. *suus*. *Vet. Dermatol.* 4: 123–131.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254.
- Currie, B. J., G. P. Maguire, and Y. K. Wood. 1995. Ivermectin and crusted (Norwegian) scabies. *Med. J. Aust.* 163: 559–560.
- Davis, D. P., and R. D. Moon. 1990. Density of itch mite, *Sarcoptes scabiei* (Acari: Sarcoptidae) and temporal development of cutaneous hypersensitivity in swine mange. *Vet. Parasitol.* 36: 285–293.
- Dobson, K. J. 1981. External parasites, pp. 579–589. In A. D. Leman et al. (eds.), Diseases of swine. Iowa State University Press, Ames, IA.
- Falk, E. S., and T. J. Eide. 1981. Histologic and clinical findings in human scabies. *Int. J. Dermatol.* 20: 600–605.
- Falk, E. S., and R. Matre. 1982. *In situ* characterization of cell infiltrates in the dermis of human scabies. *Am. J. Dermatopathol.* 4: 9–15.
- Head, E. S., E. M. MacDonald, A. Ewert, and P. Apisarnthanarax. 1990. *Sarcoptes scabiei* in histopathologic sections of skin in human scabies. *Arch. Dermatol.* 126: 1475–1477.
- Hejazi, N., and A. H. Mehregan. 1975. Scabies-historical study of inflammatory lesions. *Arch. Dermatol.* 111: 37–39.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*. 227: 680–685.
- Mellanby, K. 1944. The development of symptoms, parasitic infection and immunity in human scabies. *Parasitology*. 35: 197–206.
- Morgan, M. S., and L. G. Arlan. 1994. Serum antibody profiles of *Sarcoptes scabiei* infested or immunized rabbits. *Folia Parasitologica*. 41: 223–227.
- Morgan, M. S., L. G. Arlan, and S. A. Estes. 1997. Skin test and radioallergosorbent test characteristics of scabietic patients. *Am. J. Trop. Med. Hyg.* 57: 190–196.
- Reunala, T., A. Ranki, T. Rantanen, and O. P. Salo. 1984. Inflammatory cells in skin lesions of scabies. *Clin. Exp. Dermatol.* 9: 70–77.
- Sheahan, B. J. 1975. Pathology of *Sarcoptes scabiei* infection in pigs. I. Naturally occurring and experimentally induced lesions. *J. Comp. Pathol.* 85: 87–95.
- Stemmer, B. L., L. G. Arlan, C. M. Rapp, M. S. Morgan, and P. F. Moore. 1996. Characterization of antigen presenting cells and T-cells in progressing scabietic skin lesions. *Vet. Parasitol.* 67: 247–258.
- Young, P. R. 1989. Enhancement of immunoblot staining using a mixed chromogenic substrate. *J. Immunol. Methods*. 121: 295–296.

Received for publication 26 June 2003; accepted 17 September 2003.