Detection of IgG and IgM Antibodies to Purified Keratinolytic Proteinase in Sera from Patients with Dermatophytosis by Enzyme-Linked Immunosorbent Assay

Kwang Hoon Lee, M.D., Yang An Kim, M.D., Min Geol Lee, M.D., Jung Bock Lee, M.D.

Department of Dermatology, Yonsei University College of Medicine, Seoul, Korea

Serum samples from patients with dermatophytosis and those from controls were tested by enzyme-linked immunosorbent assay (ELISA) for detecting specific antibodies of the IgG and IgM classes against purified keratinolytic proteinase from *Microsporum canis*. Of the 130 sera tested from patients with dermatophytosis, 71 showed reactive results in the ELISA to antihuman IgG antibodies (IgG-ELISA) and 38 in the ELISA to antihuman IgM antibodies (IgM-ELISA). Of the 50 sera from controls, who were free from mycotic infection, and consisted of healthy blood donors and dermatological outpatients, 47 showed nonreactive results in the IgG-ELISA and 49 in the IgM-ELISA.

There were significant differences in specific IgG and IgM antibody reactivities according to the duration of infection. That is, the specific IgG reactivity tended to be increased in cases with a longer duration of infection but IgM reactivity increased with a shorter duration of infection. (Ann Dermatol 1:1–5, 1989)

Key Words: Dermatophytosis, ELISA, IgG and IgM antibodies, Keratinolytic proteinase

Although numerous serologic studies have demonstrated the presence of circulating antibodies to dermatophyte antigens in dermatophytosis, their clinical significance is still unknown.[1-5] In a previous study, we demonstrated that antibodies to purified keratinolytic proteinase (KPase) were detected in 75% of the sera from guinea pigs infected with *Microsporum canis (M. canis)*.[6] The immunoglobulin (Ig) class-specific antibody reactivity to purified trichophytins was examined in dermatophytosis using an enzyme-linked immunosorbent assay (ELISA) method.[7,8] However, there is little information on the difference between IgG and IgM antibody reactivities in patients with different clinical dermatophyte infections.

In the present study, the ELISA was used to evaluate the presence of IgG and IgM antibodies against KPase in the sera from both acute and chronic dermatophytosis patients, and these results were compared with noninfected individuals. In addition, a relationship between the different clinical parameters and frequency of antibody reactivities was observed.

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Reprint request to: Kwang Hoon Lee, M.D., Department of Dermatology, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul, Korea

MATERIALS AND METHODS

Sera samples

The sera used in this study were divided into two groups. Group 1 consisted of 50 sera from healthy blood donors and patients with various dermatological conditions but free from dermatophytosis; Group 2 included 130 sera from patients with dermatophytosis. Diagnoses were based on both clinical and mycologic data. Of 130 sera, 105 were from patients with dermatophytosis from which the causative organism had been identified and 25 from those with dermatophytosis showing positive direct microscopy but negative culture. To evaluate the association between antibody reactivity and the duration of infection, patients with dermatophytosis were divided into 3 subgroups as follows: Subgroup 1, 44 subjects, less than one month duration; Subgroup 2, 28 subjects, 1 to 12 months of duration; and Subgroup 3, 58 sub-

jects, more than one year duration. The serum samples were obtained from Yonsei University, Seoul. None of the subjects suffered from immunologic disorders or were undergoing immunosuppressive therapy.

Antigens

The KPase derived from *M. canis* was used as an antigen. The antigen was prepared from culture filtrates of *M. canis* by ion-exchange chromatography and gel filtration as described previously.[9]

ELISA procedure

The optimal dilutions of the antigen and antihuman immunoglobulin to be used were determined by block titration. To each well, in flat-bottomed polystyrene microtiter plates (Immulon 2; Dynatech, Alexandria, VA, USA), 100 ul of 1 ug/ml antigen suspension diluted in 0.05 M sodium carbonate buffer (pH 9.6) was added and incubated at 4°C overnight. Immediately prior to use the plates were washed three times with phosphate-buffered saline (pH 7.2) containing 0.05% Tween 20 (Tween 20-PBS). The serum samples were diluted 1:10 at the first well of every plate with Tween 20-PBS containing 1% normal rabbit serum (1% NRS), followed by serial twofold dilutions with a final dilution of 1:1,280. To remove the rheumatoid factor from the procedure of the ELISA to antihuman IgM antibodies (IgM-ELISA), equal amounts of sheep antihuman IgG (Behringwerke, West Germany) and serum, which was diluted 1:5 with 1% NRS, were incubated at room temperature for 15 min. Then the 1:10 diluted serum was centrifuged for 4 min and the supernatant was used. Positive and negative control serum samples were included on every test. Plates were incubated at room temperature for 2 h. After washing three times with Tween 20-PBS, 100 ul peroxidase-conjugated rabbit antihuman immunoglobulin (IgG and IgM-DAKO Chemicals, Denmark) was added to the well, then the plates were incubated at room temperature for 1 h. The conjugates were used at the concentration of 1:1000 for IgG and 1:500 for IgM in 1% NRS. The plate was again washed with Tween 20-PBS, and 100 ul substrate solution was added. A stock solution of substrate was prepared by dissolving 50 mg *ortho*-phenylenediamine (Sigma Chemicals Co., USA) in 5 ml absolute methanol. The working substrate solution was prepared immediately before use by adding 1 ml stock solution and 0.1 ml 3% hydrogen peroxide to 99 ml distilled water.[10] The chromogenic reaction was stopped after 30 min with 25 ul 8 N H_2SO_4. The plates were then read spectrophotometrically at 490 nm on a Dynatech ELISA reader. A well containing no serum served as a blank for the assay.

RESULTS

Optical densities(ODs) in the ELISA to antihuman IgG antibodies (IgG-ELISA) and IgM-ELISA were recorded for serum samples from dermatophytic and

Table 1. Values of optical densities at 490nm in 1:160 dilution of sera from dermatophytics in relation to duration of disease and nondermatophytics by IgG-and IgM-ELISA against proteinase from *M. canis*

OD at 490nm	No. of Nondermatophytics (N=50)		No. of Dermatophytics					
			≤1M (N=44)		>1M~<1Y (N=28)		≥1Y (N=58)	
	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
0.05	19	22	7	9	1	5	4	18
0.05-0.09	28	27	18	18	14	13	15	29
0.10-0.19	2	1	15	17	10	10	33	10
0.20-0.29	1	—	3	—	3	—	6	1
0.30-0.39	—	—	1	—	—	—	—	—
No. Individuals(%) showing OD≥0.10	3(6)	1(2)	19(43)	17(39)	13(46)	10(36)	39(67)	11(19)

OD: Optical density

nondermatophytic individuals. An OD of 0.1 or above at 490 nm in a 1:160 dilution of sera was considered as the cut-off value. Table 1 shows the ODs for 1:160 dilutions of sera from the different categories of dermatophytic individuals in relation to the duration of disease and nondermatophytic individuals. Of 50 serum samples from nondermatophytic individuals, 47 or 94% were nonreactive at an OD of 0.10 in IgG-ELISA and 49 or 98% in IgM-ELISA. Of 130 serum samples from dermatophytic patients, 71 were reactive in the IgG-ELISA and 38 in the IgM-ELISA.

IgG antibody reactivity was the most frequent in subgroup 3 (6%), while IgM antibody reactivity was most frequent in subgroup 1(39%). The IgG and IgM antibody titers of sera are presented in Fig. 1, which indicate a trend toward increased IgG reactivity as the duration of infection was sustained, but an increased IgM reactivity when the duration of infection was shorter.

The results of the reactivities of IgG-and IgM-ELISA in serum samples from patients who were divided into subgroups according to different dermatophytoses are shown in Table 2. Patients with tinea pedis showed more frequent reactivities to IgG-ELISA than those with other dermatophytoses, whereas the reactivities to IgM-ELISA were the most frequent in those with tinea capitis.

Table 3 shows the results of the reactivities to IgG- and IgM-ELISA in different dermatophyte infections. *Trichophyton (T.) rubrum* infections gave a higher

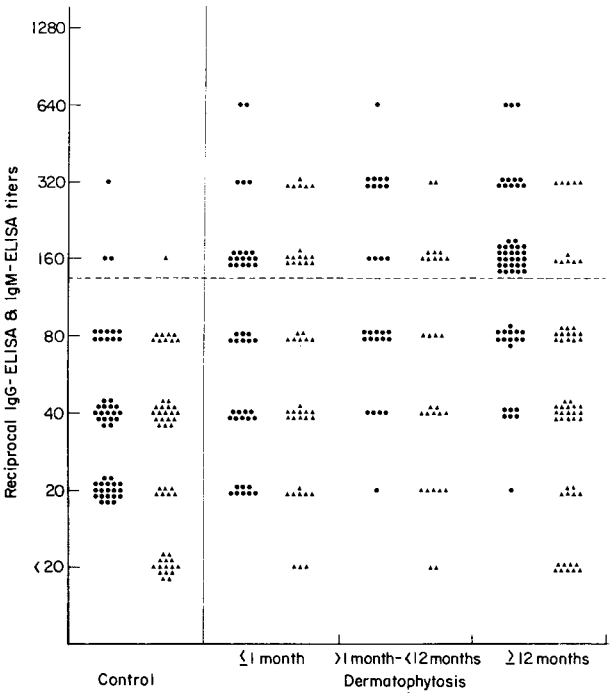

Fig. 1. Antibody titers of IgG-ELISA and IgM-ELISA to KPase from *M. canis* in sera from dermatophytics in relation to duration of disease and nondermatophytics. •: IgG Ab, ▲: IgM Ab, ---: cut-off value line.

Table 2. Reactivities of IgG-and IgM-ELISA against proteinase from *M. canis* in sera from patients with different dermatophytoses

Clinical Type	No. of Individuals	No. Reactive (%)	
		IgG	IgM
Tinea capitis	13	2(15)	6(46)
Tinea corporis	17	7(41)	7(41)
Tinea cruris	25	15(60)	6(24)
Tinea pedis[a]	75	47(63)	20(27)
Nondermatophytics	50	3(6)	1(2)

[a]Four patients with tinea manus and four patients with tinea unguium were included in this category.

Table 3. Reactivities of IgG-and IgM-ELISA against proteinase from *M. canis* in sera from patients infected with different dermatophytes

Infection with Dermatophytes	No. of Individuals	No. Reactive(%)	
		IgG	IgM
T. rubrum infection	78	43(55)	24(31)
T. mentagrophytes infection	10	5(50)	3(30)
M. canis infection	15	3(20)	6(40)
Nondermatophytics	50	3(6)	1(2)

rate of reactivity to IgG-ELISA than both *T. mentagrophytes* and *M. canis* infections, while the reactivity to IgM-ELISA was the highest in *M. canis* infections.

DISCUSSION

The sera samples from 55% of the patients with dermatophytosis were reactive to IgG-ELISA and 29% were reactive to IgM-ELISA. However, of 50 patients without dermatophytosis 3 showed reactivity to IgG-ELISA and 1 to IgM-ELISA. Mean IgG and IgM values showed significant differences between patients with dermatophytosis and nondermatophytic individuals (data not shown). With respect to its capacity to form a circulating antibody, the cross-reactivities between KPases of dermatophytes have not been defined. In the present study, although we did not correlate the reactivity to KPase with that to other antigens, more than half of the control group were selected from outpatients who had other skin diseases. Therefore, these results suggest that the antibodies specific for dermatophytes could be produced by KPase in human dermatophytosis.

The IgG and IgM antibody reactivities were generally quite different according to the clinical status of dermatophytosis and closely correlated with those in our study. IgG reactivity increased when the duration of the infection was long but IgM reactivity increased as the duration of infection shortened. Among the dermatophytes isolated in this study, *T. rubrum* was primarily isolated from patients with tinea pedis and tinea cruris or chronic dermatophytosis, while *M. canis* was mainly isolated from patients with tinea capitis or acute lesions. Also patients with *T. rubrum* infections or tinea pedis and tinea cruris showed the highest IgG reactivity, whereas those with *M. canis* infections or tinea capitis showed the highest IgM reactivity. These results may be related to the established fact that the geophilic and zoophilic species are more likely to produce acute inflammatory lesions or tinea capitis, while anthropophilic species are more often isolated from chronic noninflammatory infections or tinea pedis and tinea cruris.[11]

The host response between patients with acute and chronic dermatophyte infections is different.[2,11,12] It is well known that cell-mediated immunity (CMI) plays a role in the host defense against dermatophytes in acute dermatophytosis as reported by a number of studies.[2,11,12,13] Chronic infections usually have poor or absent CMI responses and persist for a long time regardless of treatment.[14,15] However, the exact mechanism which is related with the diminution of CMI is not known. The antiidiotypic antibody is capable of leading to selective elimination or inactivation of the T and B lymphocytes which carry the relevant idiotypic receptor.[16-18] Ahmed[12] assumed that if the antidermatophyte antibody would behave like an antiidiotypic antibody, it would block the T-cell receptor resulting in diminished cellular response and finally resulting in chronic dermatophyte infection. However, there is no evidence to suggest the presence of such type of antibodies. Our results showed that IgG antibody reactivities were increased in proportion to the duration of infection, and it suggests that these antibodies may be related to the persistence or chronicity of the infection.

In conclusion, the reactivity of IgG-ELISA against KPase in dermatophytosis seems to be partly useful in clinical practice, and IgG and IgM antibody reactivities were significantly different between acute and chronic dermatophytosis patients. The biologic role of these antibodies on the diminution of CMI in chronic dermatophytosis still remains a subject to be investigated.

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