HUMORAL ANTIBODY RESPONSE IN DERMATOPHYTOSES

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Summary

Humoral antibody response in patients suffering from dermatophytoses and in normals is described. Different fractions of antigen - (trichophytin) were prepared and used to detect circulating antibodies in patients and in normals by gel precipitation test and complement fixation test. Proctor's method was found to be superior to Ouchterloney's method for gel precipitation test. Preciptins were detected in more sera and in higher titre than complement fixing antibodies. There was no significant difference in titre of sera of patients and normals. The specificity of these antibodies is discussed.

Reports in the literature regarding humoral antibody response in dermatophytoses are conflicting. Reviewing this subject Kligman and Delamter[1] expressed the view that classical circulating antibodies in infected human beings are not convincingly demonstrated. Tomomatsu [2,3] has demonstrated circulating antibodies in immunized animals and in humans by complement fixation and gel precipitation techniques. Reyes and Friedman[4] detected antibodies in sera of patients by passive haemagglutination test with sheep erythrocytes coated with mycelial extract. Recently Grappel et al[5] have discussed this subject in detail. They are of the opinion that inspite of the superficial nature of the infection, circulating antibodies have been demonstrated in sera from both animals and humans with natural or experimental dermatophytoses. The present paper reports our attempts to demonstrate circulating antibodies against dermatophytes in human sera and discuss the specificity of such antibodies.

Material and methods

The antigen was prepared from mycelial matt as well as from cellfree culture broth. It was prepared from fluffy as well as late pigment producer variety* of T. rubrum.

T. rubrum was grown in Keeney and Erikson's[6] medium. Two sets of such medium were prepared,each consisting of 25 Erlenmeyer flasks containing 100 mls of sterile medium.

One set was inoculated with fluffy variety of T. rubrum while the other set was inoculated with late pigment producer variety of T. rubrum. Both the sets were processed similarly but separately throughout. Flasks were incubated at room temperature for one month to obtain a thick pelical matt of fungus growth. The broth culture was then poured off and processed separately for the extraction of trichophytin.

* Late pigment producer variety is a new variety of T. rubrum. According to our knowledge this variety has not been described in the literature so far. Details of this variety will be described in a separate paper.

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Trichophytin from cellfree culture broth

Trichophytin was prepared from cellfree culture broth by modifying the method described by Reyes and Friedman.[4] The cellfree culture broth was concentrated to 1/100th of volume by natural evaporation and centrifuged. The supernatant was seitz filtered and mixed with an equal volume of cold and concentrated acetone. The resulting precipitate was dissolved in distilled water and then diluted to obtain final concentration of 0.1 percent. These fractions were designated as F_1 (First fraction from fluffy variety) and L_1 (First fraction from late pigment producer variety). The supernatant from above was then precipitated with ten volumes of cold concentrated acetone and was purified and diluted in a similar manner. These fractions were designated as F_2 (second fraction from fluffy variety) and L_2 (second fraction from late pigment producer variety.)

Trichophytin from mycelia

Trichophytin was extracted from mycelia by following phenol water extract method as described by Tauber and Garson[7] with some modifications. Washed sterile mycelial mass was disintegrated and equal volume of sterile distilled water was added to it, to obtain a homogenous mixture. It was then mixed with an equal volume of concentrated carbolic acid and kept in cold for three hours, and centrifuged. This resulted in separation of three phases. The upper aqueous phase, intermediate mycelial phase and lower phenol phase. The mycelial phase was discarded while the other two were separated and processed in sterile conditions.

The aqueous phase and the phenol phase were precipitated separately by adding ethanol. The precipitate was purified and diluted as mentioned in previous method. The fractions obtained were designated as Af_1 and Al_1 (Aqueous fraction from fluffy variety and aqueous fraction late pigment producer variety) and Pf and Pl (Phenol fractions from fluffy and late pigment producer variety). Thus totally eight different fractions were obtained viz. F_1, L_1, F_2, L_2, Af_1, Al_1, Pf and Pl.

In order to detect the presence of humoral antibodies, gel precipitation test and complement fixation test were carried out. Sera of 35 patients and 20 normals were tested.

Gel precipitation test

It was carried out by two different methods (1) Ouchterloney's method[8] and (2) Proctor's method[9] with some modifications. In Ouchterloney's method[8] for each fraction of antigen one slide (3"x2") containing two sets, each of three horizontal rows, each row having eight wells were used. In each set the first and the third row were used for antigen, while the central row was used for serum. In proctor's method[9] the composition of agar and arrangements of wells were different. There was a central serum well of 6 mm diameter, surrounded by four small antigen wells of 3 mm diameter at a distance of 6 mm from the central well. In one slide (3"x2") totally two serum wells and eight antigen wells were bored. For each fraction of antigen one slide was used, thus totally eight slides were used for one serum sample.

Complement fixation test

Complement fixation test was carried out according to Wyler's method[10]. For the test equal amount of each fraction of antigen was mixed and diluted with saline to make the antigen mixture isotonic. This antigen mixture was anticomplementary, hence for each test, compensated complement dilution was used.

TABLE 1

Results of Serological Tests

	Total	Gel Precipitation				Complement Fixation			
		Positive		Negative		Positive		Negative	
		No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage
Patients	35	22	62.85	13	37.15	17	48.6	18	51.4
Normals	20	8	40	12	60	6	30	14	70

TABLE 2

Serum titre of patients and normals.

Complement Fixation

Serum Titre	Patients		Normals	
	No.	%	No.	%
Direct	8	47.07	3	50
1 : 2	5	29.42	2	33.33
1 : 4	1	5.89	1	16.67
1 : 8	2	9.37	—	—
1 : 16	1	5.89	—	—
1 : 32	—	—	—	—
1 : 64	—	—	—	—
Total	17	—	6	—

GEL Precipitation

Serum Titre	Patients		Normals	
	No.	%	No.	%
Direct	5	22.73	2	25
1 : 2	5	22.73	3	37.5
1 : 4	4	18.2	2	25
1 : 8	3	13.63	—	—
1 : 16	3	13.63	1	12.5
1 : 32	1	4.55	—	—
1 : 64	1	4.55	—	—
Total	22	—	8	—

Discussion

Analysis of results shows that precipitins were detected in 62.85% of patients and 40% of normals. The titre in both the groups ranged from nil to 1: 64.

All different fractions of the antigen gave positive results in similiar titre. F_1, L_1, F_2, L_2, Af_1, and Al_1 gave comparatively broad and diffused precipitin bands while Pf and Pl (Phenol fractions) gave comparatively thin and sharp bands indicating these fractions were chemically different than others.

Precipitin bands given by antigenic fractions prepared from fluffy variety did not differ from that of late pigment producer variety, and both the groups of antigen gave same titre for each sample, indicating that there was no immunological difference in the fluffy and the late pigment producer variety. Ouchterloney's method[8] is most commonly used for gel precipitation and was found to be satisfactory for that purpose. Proctor's method[9] is comparatively uncommonly used and meant specially for antigens prepared from fungi. Both the methods gave same titre for each of the samples. The borate buffer used in Proctor's method makes the agar gel more transparent and clear and therefore precipitate can be easily seen in the agar. However, the fact that it is self sterilizing makes it a very good medium for routine use.

Proctor's method[9] has not been widely used. Most of the workers use Ouchterloney's method[8]. In the present study the superiority of Proctor's method was convincingly felt.

Complement fixing antibodies were detected in 48% of patients and 30% of normals. Sera of two patients showed anticomplementary effect. The titre in both the groups ranged from nil to 1:16.

Titre of complement fixing antibodies was low and they were detected from less sera as compared to precipitins.

There was no significant difference in titres of sera of persons suffering from dermatophytoses and that of normals. The low titre in subjects with active ringworm could be due to the fact that antigens of dermatophytes remain localized in the superficial layers of the skin and fail to reach the major sites for antibody production or that dermatophyte reacting antibodies may be only cross-reacting antibodies formed in response to antigenically related agents and not due to dermatophytes themselves. It could also be due to technical difficulties, since preparation of standardized antigens from mould is difficult and such antigen may lack the sensitivity required to give the result in test tube. The presence of antibodies in normal persons suggest that dermatophyte reacting antibody in human sera is not specific for dermatophytes. It may be that some organisms constantly found in our atmosphere and which share a common antigen with dermatophytes come in contact with man resulting in antibody production. We did not attempt to study the specificity of these antibodies; but Pepys et al[11] demonstrated pricipitins in 50% of sera of patients with T. rubrum infections which not only reacted with mycelial extracts and culture filtrates of T. rubrum and T. mentagrophytes, but cross-reacted with antigens of Cladosporium herbarum. Penicillium notatum, and Aspergillus fumigatus. Reyes and Friedman[4] detected antibodies in sera of patients by passive haemagglutination tests with sheep erythrocytes coated with mycelial extracts. These antibodies also reacted with antigens of species of Pencillium and Hormodendrum and could be completely absorbed with mycelia of these saprophytes. This hypothesis of nonspecificity of dermatophytes reacting antibodies was further supported by the findings of Walzer and Einbinder[12] who by means of indirect fluorescent antibody technique demonstrated that antibodies to dermatophytes are nonspecific and are directed against an antigen common to many organisms.

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