A COMMON ANTIGEN BETWEEN CANDIDA ALBICANS AND MYCOBACTERIUM TUBERCULOSIS

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Contrary to other infectious diseases, the antibody production *in vivo* is very low in candidiasis. Healthy individuals harbor *Candida albicans* saprophytically, and therefore has serologically less specificity.

As an immune reaction for *Candida*, agglutination test has generally been used. However, it tends to give a spontaneous agglutination¹⁾²⁾. It is also known that a cross agglutination would occur between *Candida* and bacteria strains or among *Candida* strains themselves²⁾³⁾⁴⁾. Akiba⁵⁾ and Ata⁶⁾ reported that a cross reaction was observed between *Candida albicans* and *Mycobacterium tuberculosis*. A series of experiments was therefore carried out to study a common antigen for tubercle bacillus and *Candida albicans*.

EXPERIMENT I

Method. Immunization was carried out in the following way: *Candida albicans* was cultured on the Sabuoraud medium at 30°C for 48 hours. The grown organisms were suspended in physiological saline, 1 mg per ml, and killed by heating at 70°C for 30 minutes. Increasing doses of this suspension, from 0.1 to 1.0 mg per kg body weight, were injected into the ear vein of the rabbit every other day. The tubercle bacilli were grown in Sauton medium at 37°C for 4–8 weeks, sterilized at 100°C for 60 minutes, and then suspended. The concentration of the suspension and dosage were the same as for *Candida albicans*. The anti-*Candida* immune serum and anti-tubercle bacillus immune serum are abbreviated herein as ACS and ATS, respectively.

A simultaneous immunization against *Candida albicans* and tubercle bacillus was also attempted. These suspensions were injected into each ear vein of the rabbit every other day.

The antigen against *Candida albicans* was prepared as follows: *Candida albicans* was grown in Sabuoraud peptone medium at 30°C for 5–7 weeks. The filtrate of the medium was sterilized at 70°C for 30 minutes, and acidified to pH 5.5 with acetic acid. A 50 gm % zinc chloride solution was added gradually and under shaking until saturation, shaken for 15 minutes and then centrifuged for 10 minutes at 2000 rpm. The sediment was suspended in 1/10 volume of 20 % Na₂HPO₄ solution at 37°C for 2 hours, then left in an ice chest overnight. A brown transparent supernatant was obtained as antigen.

TABLE 2.
Agglutination of Tuberculin sensitized erythrocytes

Organisms for immunization	Candida albicans					Tubercle bacillus					Control		
Rabbit No.													
Serum dilution	1	2	3	4	5	6	7	8	9	10	11	12	13
4	+	+	+	+	+	+	+	+	+	+	-	-	-
8	+	+	+	+	+	+	+	+	+	+	-	-	-
16	+	+	+	+	+	+	+	+	+	+	-	-	-
32	+	+	+	+	+	+	+	+	+	+	-	-	-
64	+	+	+	+	+	+	+	-	-	+	-	-	-
128	+	+	+	-	+	+	-	-	-	-	-	-	-
256	-	-	+	-	+	+	-	-	-	-	-	-	-
512	-	-	-	-	+	-	-	-	-	-	-	-	-
1024	-	-	-	-	-	-	-	-	-	-	-	-	-

with the ATS and CSE, and 256X with the same serum and tuberculin. (Tables 1 and 2)

EXPERIMENT II

Absorption of group agglutinin was carried out in ACS with TSE and in ATS with CSE. The sera freed of the group agglutinin were subjected to the immune reaction.

1. Absorption in test tube

Method: A mixture of 2 cc of Candida antigen solution, 10 cc of Eagle's phosphate buffered saline and 2 cc of washed sheep erythrocytes was incubated at 37°C for 3 hours while shaking at 15 minutes intervals for sensitization. The mixture was then centrifuged at 1000 rpm for 10 minutes, the sediment was washed with Eagle's buffered saline 3 times. Two cc of thus prepared CSE was added to 1 cc of ATS, incubated for 12 hours while shaking every 2-3 hours, and centrifuged. The supernatant represented ATS absorbed by CSE. The same procedure was repeated with 0.8 cc of original tuberculin solution, 10 cc of Eagle's saline and 2 cc of washed erythrocytes to obtain TSE, and exactly the same procedure was used to prepare ACS absorbed

TABLE 3.
Agglutination in ACS after absorption with TSE

Agglutination	Agglutin titer for TSE										Agglutin titer for CSE									
Absorption	Before absorption					After absorption					Before absorption					After absorption				
Serum dilution	4	8	16	32	64	128	256	512	1024		4	8	16	32	64	128	256	512	1024	
Rabbit No. 29	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-
30	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-
31	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-
32	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-
33	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-

by TSE. Immune reactions were carried out with these sera with the following results.

Results: Table 3 shows that the agglutinin for TSE in ACS was completely absorbed by TSE and the titer for CSE was markedly reduced. Similar complete absorption of agglutinin for CSE was observed with ATS, but the reduction of titer for TSE was slight in this case. (Table 4)

TABLE 4.
Agglutination in ATS after absorption with CSE

Aggiutination	Agglutin titer for CSE								Agglutin titer for TSE																		
Absorption	Before absorption				After absorption				Before absorption				After absorption														
Serum dilution	4	8	16	32	64	128	256	512	1024	4	8	16	32	64	128	256	512	1024	4	8	16	32	64	128	256	512	1024
Rabbit No. 34	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
35	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
36	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
37	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
38	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

2. Absorption *in vivo*

Method: Rabbits immunized against *Candida* were injected subcutaneously daily with killed tubercle bacilli in the dose of 1 mg/kg for 36 days. The serum was subjected to the study. Rabbits immunized against tubercle bacilli were treated in the same manner with 10 mg of killed *Candida* per/kg.

Results: When rabbit immunized against *Candida* were treated with large doses of killed tubercle bacilli, the agglutinin titer for CSE was markedly reduced. (Table 5)

TABLE 5.
Agglutination of CSE with serum obtained from rabbit immunized against Candida albicans and given excessive doses of killed tubercle bacilli

Agglutination	Agglutinin titer for CSE												
Days	Before absorption	After absorption											
		3	6	9	12	15	18	21	24	27	30	33	36
Rabbit No. 39	128	128	64	64	32	16	32	16	32	32	8	8	16
40	64	64	32	32	16	16	32	16	8	4	4	8	32
41	64	32	32	16	8	4	4	8	32	64	16	16	32
42	32	32	16	8	4	16	32	16	16	8	8	16	16
43	32	16	8	8	16	16	32	32	16	8	16	8	16

The reverse of this showed a slight decline of the titer which increased later. (Table 6)

TABLE 6.

Agglutination of TSE with serum obtained from rabbit immunized against tubercle bacilli and given excessive doses of killed Candida albicans

Agglutination		Agglutinin titer for TSE											
Days	Before absorption	After absorption											
		3	6	9	12	15	18	21	24	27	30	33	36
Rabbit No. 44	256	256	256	128	128	128	256	128	128	256	128	128	128
45	256	256	128	128	256	256	256	256	256	256	128	256	256
46	256	128	64	128	256	128	128	256	256	128	128	256	256
47	128	128	64	128	128	256	128	256	256	256	128	128	128
48	128	128	128	256	128	128	64	128	256	256	128	128	64

EXPERIMENT III

Method

1. Antigen analysis for Candida

Dried *Candida* organisms were ground, added with 10 volumes of 0.1 N NaOH and stirred for 3 days. After centrifugation, the supernatant was dialyzed, filtered, condensed, the pH adjusted to 4.0 with 0.25 N trichloroacetic acid (TCA) and an equal volume of ethanol was added. The precipitate was washed with acetone several times and dried. The dried material was washed with TCA several times until amber supernatant became clear. The precipitate was then dialyzed overnight to remove TCA, reprecipitated with ethanol and dried after acetone washing. The material thus prepared gave negative protein reaction when studied by Biuret, ninhydrin, Millon, xanthoprotein reactions and TCA precipitation. Reactions to Fehling, Benedict and Molish reagents were equivocal.

Phenol treatment was carried out as follow : Dried *Candida* organisms were extracted with 90–95 % phenol for 3 days under agitation, filtered and the precipitate was thoroughly washed with ethanol to remove phenol. The precipitate was heated in distilled water at 100°C for one hour, dialyzed against running water for 2 days, filtered and condensed. Three volumes of absolute ethanol were added to it, sodium acetate was then added to the concentration of 1 %, left overnight, the precipitate was washed with acetone several times. The powder thus prepared was dissolved in distilled water, dialyzed against distilled water, condensed, and precipitated with 3 volumes of ethanol, the precipitate was washed with acetone several times and dried. The entire procedure was repeated 3 times.

The final material was positive to Molish and carbazole reactions, negative to Fehling test, and negative to Benedict, ninhydrin, Millon and xanthoprotein tests, no turbidity with TCA and gave a positive Sakaguchi reaction.

2. Chemical analysis of tuberculin

To the original tuberculin solution was added 50 % TCA to the final concentration of 10 % TCA. The mixture was left overnight at room temperature, centrifuged. The precipitate was dissolved in 0.1 N NaOH and the TCA precipitation was repeated several times. The final precipitate was dialyzed against distilled water overnight, reprecipitated with ethanol, washed with acetone several times and dried. The

chemical properties of the final product revealed positive protein reactions, negative sugar reactions and equivocal Molish test.

Similar chemical characterization was carried out on the 10 % TCA soluble fraction. The TCA supernatant was precipitated with 5 volumes of methanol after being left overnight. The precipitate was dissolved in a small volume of distilled water, precipitated with TCA, the supernatant was treated with methanol and the same procedure was repeated. The final precipitate was dialyzed against distilled water overnight, concentrated, reprecipitated with ethanol, and washed with acetone and dried.

The reactions this product gave were positive for sugar and negative for protein.

3. Sensitization of erythrocytes with purified antigen fractions and agglutination reaction.

The protein and polysaccharide fractions were used in 1 % solution to sensitize erythrocytes and agglutination was carried out in the method described.

Results: With the purified fraction of *Candida*, the agglutination reaction was still demonstrable although the titers were lower, and the protein fraction gave a weaker reaction than the polysaccharide fraction.

In ATS, the same but definite reaction was seen with *Candida* polysaccharide, no

TABLE 7.

Agglutination of erythrocytes sensitized with various fractions of Candida albicans

Organisms for immunization	Candida albicans					Tubercle bacillus					Control	
Rabbit No.	49	50	51	52	53	54	55	56	57	58	59	60
Sensitized antigen												
Candida albicans	128	128	256	128	64	32	64	64	32	16	0	0
Protein fraction of Candida albicans	16	8	16	8	8	0	8	4	0	0	0	0
Polysaccharide fraction of Candida albicans	64	64	128	128	32	16	16	16	8	16	0	0

TABLE 8.

Agglutination of erythrocytes sensitized with various tuberculin fraction

Organisms for immunization	Candida albicans					Tubercle bacillus					Control	
Rabbit No.	49	50	51	52	53	54	55	56	57	58	59	60
Sensitized antigen												
Tuberculin	64	64	128	64	64	512	512	128	256	64	0	0
Protein fraction of tuberculin	0	0	8	0	0	4	4	0	0	0	0	0
Polysaccharide fraction of tuberculin	64	32	64	32	16	256	128	64	64	32	0	0

demomonstrable reaction was obtained with the protein fraction. (Table 7)

Using tuberculin polysaccharide fraction, the titers were somewhat lower than with crude tuberculin, but the reaction was definite. No reaction was obtained with the protein fraction to either serum. (Table 8)

Thus, polysaccharide fraction of both antigens were antigenic to both immune sera, whereas the protein fraction of *Candida* showed antigenicity to ACS but not to ATS. Tuberculin protein demonstrated no antigenicity to either serum.

SUMMARY

1) Antigen prepared from *Candida albicans* and tuberculin showed a cross reaction with anti-*Candida* and anti-tubercle bacillus immune sera.

2) *In vitro* absorption study of the common antigen demonstrated that it constitutes a greater part of the antigenicity in the *Candida* antigen than in tuberculin.

In vivo absorption study showed that *Candida* has an antigenicity similar to tubercle bacilli.

3) The polysaccharide fraction of each antigen revealed antigenic properties in both immune sera. The protein fraction of *Candida* antigen was antigenic in anti-*Candida* serum, but tuberculin protein lacked in antigenicity.

4) These results suggest that the antigen shared by the both organisms is contained in the polysaccharide fraction of *Candida* antigen and tuberculin.

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