

Phagocytosis of *Candida albicans* by eosinophilic leukocytes

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The percentage of eosinophils phagocytosing Candida albicans spores approximates that of neutrophils in in vitro experiments. The phagocytic activity of the eosinophils is shown to parallel anti-Candida IgG serum antibody titers and to involve the complement system. By means of an immunofluorescent technique, with the use of rhodamine B conjugated to bovine serum albumin to block nonspecific staining, the presence of antibody on the phagocytosed spores is demonstrated.

The function of eosinophils in immunologic phenomena remains an enigma despite considerable investigation. Evidence has accumulated that these cells may phagocytose antigen-antibody complexes,¹⁻⁴ bacteria,^{5,6} and mycoplasma.⁷ Lehrer has recently demonstrated the ability of eosinophils to ingest and kill *Candida albicans* spores.⁸

This report confirms the ability of the human eosinophil to phagocytose *C. albicans*. In addition, the attachment of immunoglobulin G to the engulfed organisms is demonstrated by an immunofluorescent technique.

MATERIALS AND METHODS

Normal human IgG was isolated from serum by DEAE-cellulose chromatography and then digested with papain. The digested product was fractionated by chromatography on Sephadex G-200, following which starch block electrophoresis was performed. The Fc fragment thus obtained was used to immunize rabbits, with the production of a monospecific antiserum. Partly purified IgA, IgM, and IgD, isolated from myeloma sera, were used to immunize rabbits. After the animals were bled, monospecific antisera were obtained by absorption with human cord blood which had been copolymerized with glutaraldehyde. Monospecific goat antiserum to human IgE was obtained by a method previously described.⁹ These antisera were conjugated with fluorescein isothiocyanate (FITC), and the free FITC

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TABLE I. Serum anti-Candida immunoglobulin titer

Serum	IgG	IgA	Antibody titer		
			IgM	IgD	IgE
S ₀	0	1	0	1	0
S ₁	1:5	1:3	1:5	0	0
S ₂	1:729	1:1	1:9	0	0
S ₃	1:125	1:9	1:27	0	0
S ₄	1:125	ND	ND	ND	ND
S ₅	1:125	1:27	1:81	0	0
S ₆	1:125	1:9	1:3	0	0

S₀ was obtained from patient with hypogammaglobulinemia, sera S₁ to S₆ obtained from 6 other random donors with no evidence of infection.

ND = not done.

was removed by filtration through Sephadex G-25. The conjugates were then passed through DEAE cellulose, eluted with 0.05M phosphate buffer at pH 6.3, concentrated, and adjusted to $\frac{1}{4}$ unit for use.¹⁰ Bovine serum albumin (BSA) was conjugated with rhodamine B in a similar manner. Commercial (Hyland Labs., Irvine, California) anti- β_2 C conjugated with FITC was used for detection of β_2 C attachment to the antigen-antibody complexes.

Sera were obtained from 6 apparently healthy donors and from one patient with adult onset hypogammaglobulinemia. None of these donors had evidence of recent or past infection by *C. albicans*. *C. albicans* grown in Sabouraud-dextrose broth was washed 3 times in saline and a suspension prepared containing 1.0×10^6 spores per milliliter. Smears of the organisms were fixed with cold acetone and then overlaid with serial dilutions of the sera and incubated for 30 minutes. The smears were then stained with the FITC-labeled monospecific antihuman immunoglobulins. The last serum dilution yielding any positive staining of organisms was considered the anti-Candida antibody titer.

Heparinized blood was obtained from 3 individuals with eosinophilia of 8 to 24 per cent and allowed to sediment for one hour at room temperature. The leukocytes were washed 3 times with Tris buffer containing magnesium, calcium, and human albumin (Tris ACM). Cell counts of the final suspensions were adjusted to 1.0×10^6 per milliliter with Tris ACM. Mixtures containing 0.1 ml. of white cells, 0.1 ml. of organisms, and 0.3 ml. of serum were incubated at 37° C. for 15 minutes, centrifuged, and washed 3 times with Tris ACM. In some experiments the sera were heated to 56° C. for 30 minutes before incubation. After fixation with cold acetone, hematoxylin and eosin and Gram stains were made.

For the immunofluorescent experiments, separate smears were fixed with cold acetone for one minute and then stained with the rhodamine B-BSA conjugate for 20 minutes to eliminate nonspecific binding of the FITC conjugates.¹¹ After having been washed with phosphate-buffered saline (PBS), the smears were overlaid with the FITC-antimmunoglobulin conjugates for 30 minutes, again washed with PBS, and finally mounted with non-fluorescein-buffered glycerine. The smears were examined under oil immersion, and 1,000 eosinophils and neutrophils were counted to determine the percentage of each containing organisms.

RESULTS

The anti-Candida titers determined by indirect immunofluorescence for each immunoglobulin class are listed in Table I. The serum with no detectable IgG titer was from the patient with hypogammaglobulinemia. As would be expected from other studies,¹² the sera from the 6 other subjects contained significant titers of IgG and relatively low titers of IgA and IgM antibodies despite the fact these individuals lacked evidence of Candida infection.

The results of the phagocytosis experiments with 3 of the sera (S₁, S₂, S₃)

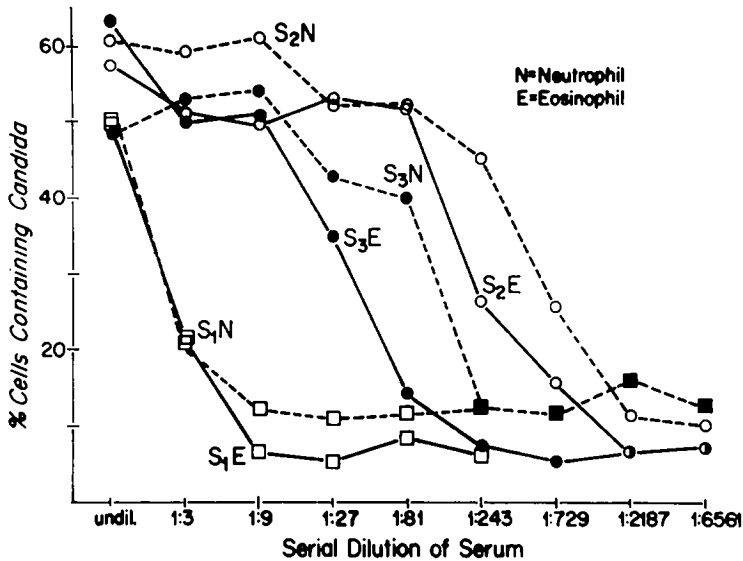


FIG. 1. The parallel relationship of serum anti-*Candida* IgG titers and phagocytosis of spores by eosinophils and neutrophils is demonstrated. Titer of serum S₁ is 5; S₂, 729; and S₃, 125. □ = serum 1; ○ = serum 2; ● = serum 3; N = neutrophils; E = eosinophils.

are presented graphically in Fig. 1. By comparing these results with the antibody titers recorded in Table I, it can be seen that there is a correlation between the degree of phagocytosis and the anti-*Candida* IgG titers. Such an association is not apparent with IgA and IgM. When serum was not incubated with organisms or when the serum lacking antibody (S₀) was used, the percentage of eosinophils containing organisms did not exceed 13.5, and the percentage of neutrophils did not exceed 12.5. This low degree of phagocytosis is probably accounted for by the inability to completely wash off antibody from the donor leukocytes, for the fluorescent staining revealed IgG antibody attached to phagocytosed organisms. Over all, when incubated with the 6 sera containing IgG antibody, the average number of eosinophils containing organisms was 55.8 per cent, closely approximating the number of neutrophils (51.5 per cent). Heating the sera at 56° C. for 30 minutes before incubation decreased the degree of phagocytosis three- to sixfold. In every preparation, almost all *Candida* organisms seen within cells stained positively with the FITC-anti-IgG conjugate (Fig. 2). The great majority, but not all, of phagocytosed spores stained with the anti-β₁C conjugate.

DISCUSSION

Phagocytosis of *C. albicans* spores by human eosinophils is clearly demonstrated in this study, and the percentage of eosinophils engaging in phagocytosis closely approximates that of the neutrophils. Two indications of the importance of antigen-antibody complexes in this phenomenon are noted. The first is that almost all organisms seen within eosinophils stained with rabbit anti human IgG conjugated with FITC. Secondly, the ability of serum to enhance

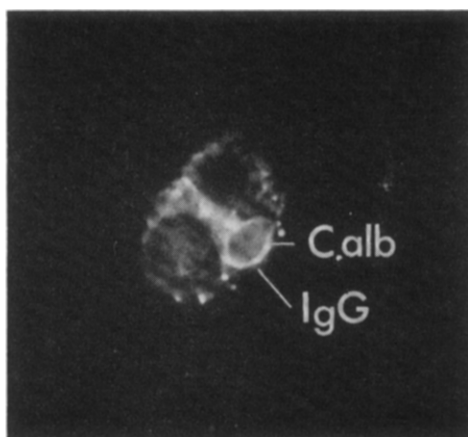


FIG. 2. The attachment of IgG antibody to a *C. albicans* spore contained within an eosinophil is shown by immunofluorescent technique.

phagocytosis was abolished when it was diluted beyond the IgG antibody titer as determined by indirect immunofluorescence. In the present studies there was no correlation between phagocytosis and the titers of the other immunoglobulin antibodies. It might be speculated that if a serum were tested which had a higher IgM than IgG anti-*Candida* titer, the phagocytic activity of the cells might parallel the IgM titer. The probability that at least the first 4 components of the complement system are involved in the phagocytic process is indicated by a three- to sixfold decrease in phagocytosis following heating of the sera to 56° C. for 30 minutes and by the positive anti- β_1C staining of the phagocytosed spores.

To ensure that the positive staining with the FITC-conjugates truly indicates the presence of immunoglobulins in eosinophils, a technique which blocks the nonspecific binding of the FITC to the cell granules is necessary. Panush and associates¹³ used Evans blue dye for this purpose. We have used rhodamine B, a dye with a chemical structure similar to those of eosin and FITC, to cover the nonspecific binding sites of the granules before the specific conjugates were applied. Preliminary studies showed that with a blue filter in the microscope, the granules of the eosinophil would stain brown with the rhodamine B and then would not stain green when exposed to FITC. The rhodamine was conjugated to BSA to allow for saturation of nonspecific binding of protein to the cells before the FITC conjugates were used. With the use of this procedure, the granules of the eosinophil stained brown, while the spores within the cells stained green, indicating the presence of IgG or β_1C .

Previous studies using soluble antigens have shown that eosinophils may phagocytose antigen in the presence of antibody but not antigen or antibody alone.^{2, 4, 11} In the present study using particulate antigen and an immunofluorescent technique, we have directly demonstrated the presence of antigen-antibody complexes in eosinophils. The technique described should prove valuable in studying the phagocytic activity of the eosinophil toward a variety of antigen-antibody complexes.

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