Candida-Reacting Antibody in the Serum of Patients with Lymphomas and Related Disorders

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With the technical assistance of Bettina McIlroy

The objectives of this study were to determine the effect of neoplasia and various forms of cancer therapy on the production of serum antibody reactive with the yeast form of Candida albicans and to determine the role of this antibody in the pathogenesis of Candida infections. It has been clearly established that neoplasia and antimetabolite therapy depress de novo synthesis of antibody produced in response to specific antigen challenge and diminish homograft rejection. It was speculated that similar mechanisms might reduce circulating antibody reactive with monilia to the extent that there would be increased susceptibility to monilial infections. During the past 12 years the occurrence of disseminated fungus disease in patients with lymphomas and related disorders has increased in incidence. Much of this may be explained on the basis of increased awareness and better methods of detection, but it is probable that some of this increase is the result of widespread use of antimetabolites, antibiotics and adrenal cortical steroids. The fungi capable of producing systemic disease in patients with lymphomas and other neoplastic disorders include Aspergillus, Cryptococcus, Histoplasma, Mucor and Candida. In this particular study Candida albicans was selected because of its prevalence, simple growth requirements and clinical importance.

The quantity of antibody reacting with the yeast form of Candida albicans was measured in the serum of normal subjects and patients with neoplastic disease. The patients were selected from a wide age group in order to determine the prevalence of this serum factor. The immune-adherence technic was used for the detection of antibodies to Candida albicans. Immune adherence involves the attachment of antigen particles to the primate red cell surface in the presence of antibody and complement. The adherence of a particulate antigen to the surface of the red cell may be visualized microscopically or may be detected macroscopically by the formation of red cell floccules.

Materials and Methods

Experimental subjects.—Quantitative assay for antibody reactive with Candida albicans was performed on the sera from a total of 85 patients in the following categories:

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NORMAL. Two groups were selected. The first consisted of 28 newborns, infants, children and preadolescents. The adult group of 10 subjects ranged in age from 20 to 35 years. It was composed of laboratory personnel, medical students and house staff members.

LYMPHOMA AND LEUKEMIA. This group included 26 patients in various stages of illness. Most of the patients were studied before, during and after treatment (ionizing radiation, chlorambucil, nitrogen mustard, busulfan, 6-mercaptopurine and adrenal cortical steroids).

DISSEMINATED CANCER. The eight patients in this group all had far advanced, terminal malignancies.

MISCELLANEOUS. This group consisted of 13 anemic patients. There were 5 with iron deficiency anemia, 1 with disseminated lupus erythematosus, 1 with thalassemia minima, 1 with post-gastrectomy pernicious anemia, 1 with Laennec’s cirrhosis and 1 with azotemia due to chronic pyelonephritis.

Preparations of reactants.—ANTIGEN. The *Candida albicans* was freshly isolated from an active case of oral moniliasis. The organism was grown on Sabouraud’s media at room temperature for less than 48 hours. The monilia were removed from the agar slant by adding 5 to 10 ml. of normal saline to the culture tubes and agitating until a cloudy suspension developed. This suspension was transferred to a centrifuge tube and spun at 3000 rpm for 10 minutes. The sediment then was washed three times with gelatin Veronal buffer* and made up to a 2 per cent solution in this buffer. This antigen suspension was used in dilutions of from 1:30 to 1:480 in the reaction mixtures.

ANTIBODY. Blood was collected in acid-cleaned glassware and allowed to clot. The serum was separated and heat inactivated at 56 C. for 30 minutes. Unless used immediately serum samples were stored at —35 C. between determinations. Serial dilutions of test serum in gelatin veronal buffer ranging from 1/4 to 1/4096 were used for assay.

ERYTHROCYTE INDICATOR SYSTEM. Human, type O, Rh negative red cells were collected and stored as whole blood in an equal volume of modified Alsever’s solution.† They were refrigerated at 4 C. and remained suitable for use for approximately 1 month. Before use the erythrocytes were washed three times with buffer and made up to a concentration of 2 per cent in gelatin veronal buffer. The final concentration was adjusted so that 1 ml. of suspension yielded an optical density of .390 when diluted 1/10 with water and examined at a wavelength of 541 nm and a slit opening of .02 on a Beckman model DU spectrophotometer.

COMPLEMENT. A normal subject of blood type AB with an unusually low antibody dilution titer to Candida of 1/4 was selected as the source of complement. At a volume dilution of 1/40, which was kept constant in every assay, this serum provided an adequate source of complement in immune-adherence without any detectable Candida-reacting antibody. Aliquots of undiluted fresh serum were stored in dry ice until used.

Immune-adherence assay technic.—Kontes hemagglutination tubes containing 0.25 ml. of antigen (*Candida albicans*) in dilutions ranging from 1/30 to 1/480 were arranged in test tube racks in ice. Serum in 0.25 ml. amounts in dilutions of from 1:4 to 1:4096 were added slowly to the chilled antigen so that mixtures containing progressive dilutions of each were obtained. These mixtures were incubated in a water bath at 37 C. for 15 minutes after which 0.5 ml. of chilled complement (1:40 dilution of AB serum) was added to each tube. Incubation of this mixture was continued in the water bath at 37 C. for an additional 15 minutes with occasional shaking by hand. The tubes were removed from the water bath and to each 1.0 ml. of the mixture, 0.1 ml. of erythrocyte indicator solution (standardized human, type O, Rh negative red cells) were added and mixed with each tube. Incubation of the tubes was continued for 40 minutes in a water bath at 37 C. at the end of which

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*Veronal buffer, 200 ml.; calcium chloride, (.03 M) 2.5 ml.; magnesium sulfate, (.15 M) 1.5 ml.; gelatin, 1 Gm. dissolved in 100 ml. boiling distilled water; distilled water to make 1000 ml.

†Glucose, 2.05 Gm.; sodium citrate, 0.80 Gm.; sodium chloride, 0.42 Gm.; distilled water to make 100 ml.; adjust to pH 6.1 with 5 per cent citric acid; sterilize by glass filter and store at 4 C.
the degree of immune-adherence in each tube was determined by the intensity of hemagglutination. A positive reaction consisted of macroscopically discernible floccules of erythrocytes covering the bottom of the tube without a discrete ring of sedimented red cells. A smooth ring of red cells at the bottom of the tube, in the complete absence of floccule formation, indicated a negative reaction. The intensity of the reaction was graded from 4+ to negative, as illustrated in figure 1. Microscopic confirmation of immune-adherence was determined by dark field microscopy. The technic consisted of replacing the supernatant with an equal volume of distilled water and, after gently mixing, a wet preparation was made with a drop of this suspension. In a positive test the yeast form of *Candida albicans* was seen to be firmly attached to the surface of the red cells (fig. 2). During the initial stages of this study microscopic confirmation was carried out frequently, but later was found unnecessary.

**Antibody characterization.—Antibody absorption studies.** Six human serum samples with high antibody titers to *Candida albicans* were absorbed twice at 37 C. with an equal volume of the packed yeast form of Candida. The absorbed sera were assayed for antibody as outlined above.

**Antibody stimulation studies.** The quantity of antibody reacting with *Candida albicans* was determined in the serum of several normal albino rabbits. A freshly prepared suspension of monilia was killed by incubating at 65 C. for 2 hours. A 2 per cent suspension of these organisms in gelatin veronal buffer was injected intravenously at weekly intervals into each rabbit for a period of 3 weeks. Ten days after the last injection serum from each animal, obtained from venous blood, was collected and assayed for antibody reacting with

Fig. 1.—Schematic drawing of the Kontes hemagglutination tubes as viewed from below. The finely stippled areas represent diffuse homogeneous red cell floccule formation covering the bottom portion of the tubes. As the reactivity becomes less intense, the red cells have a tendency to begin to sediment out until they eventually form a smooth button, indicating a negative reaction.

Fig. 2.—Photomicrograph (× 420) taken under dark-field illumination showing the monilia as luminescent, solid spherules attached to the periphery of the red cell shadows.
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*Candida albicans* by the immune-adherence technic. At no time throughout the immunization period did the rabbits appear ill.

**CROSS REACTION STUDIES.** One ml. aliquots of serum from 5 human subjects containing a known amount of Candida-reacting antibody were absorbed twice at 37 C. with packed, washed rice starch. The quantity of rich starch varied from .5 to 2 Gm. Candida-reacting antibody then was determined on each of the absorbed serum samples by the immune-adherence technic.

**SERUM FRACTIONATION STUDIES.** Candida-reacting antibody was determined in dilutions of pooled commercial gamma globulin and on 1 patient with known hypogamma-globulinemia.

**RESULTS**

*Experimental subjects.*—NORMAL GROUP. Serum antibody titers on cord blood invariably were high, and could be titrated out to 1/4096 or higher. After the first 2 months of life infant blood showed a considerably decreased reactivity. The antibody titer on serum samples from children ranging in age from 3 months to 14 years showed variations in the low range from 1:4 to 1:512, with higher titers generally being noted in older children. A similar trend relating increased serum antibody reactivity to age was noted in the samples obtained from normal subjects in the 20 to 35 year old age group. Practically all of the individuals who were 40 years or older had high serum antibody titers. They reacted at dilutions of 1/512 or higher and practically all were over 1:4096 (fig. 3). There appeared to be no relationship between antibody reactivity and either sex or a history of previous Candida infection.

LYMPHOMA AND LEUKEMIA GROUP. There was no apparent difference in amount of Candida-reacting antibody in patients with lymphoma and leukemia as compared to normal subjects of the same age. The lymphoma group included subjects with localized or disseminated moniliasis, many of whom were terminally ill and who died within a few days after the serum was collected. Serum from a patient with acute monocytic leukemia and disseminated moniliasis reacted in a dilution of 1:4096. Two patients in this group were under 20 years of age, and they had amounts of serum antibody comparable to that of normal subjects in the same age group (table 1). These patients were or previously had been under treatment. Serial determinations on the sera of these patients during therapy showed no decrease in antibody titer (table 2).

DISSEMINATED CANCER GROUP. All the patients with advanced metastatic carcinoma showed serum Candida reactivity in dilutions of 1/4096 or greater.

MISCELLANEOUS GROUP. All patients had Candida-reacting antibody in serum dilutions of 1:4096 or greater.

**Antibody characterization studies.**—CANDIDA ABSORPTION. Following absorption of the six sera of known reactivity with the yeast form of *Candida albicans* antibody reacting to Candida was completely removed. Antibody titers dropped from 1:4096 to zero.

ACTIVE IMMUNIZATION. Inoculation of rabbits with heat-killed suspensions of *Candida albicans* increased serum reactivity from dilution to 1:128 to greater than 1:4096.

CROSS ABSORPTION. In none of the five sera tested was there reduction in antibody titer following absorption with any of the concentrations of rice starch.

SERUM FRACTIONATION. Commercial pooled gamma globulin showed Candida
antibody reactivity in a dilution of 1:512. The serum obtained from 1 adult subject with known hypogammaglobulinemia revealed Candida antibody reactivity only in a dilution of 1:32, far below that ordinarily found in the serum of normal subjects of a similar age.

DISCUSSION

The technic of immune-adherence has received relatively little attention as a method for titration of antibody in human serum. It is believed that it is about 5 to 10 times as sensitive as the usual complement fixation test and that it is particularly suited to those studies in which the end point of an antigen antibody reaction is not clear-cut.
Table 1.—Titers of Serum Antibody Reacting with Candida Albicans in 26 Patients with Lymphomas and Related Disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>Age</th>
<th>Duration of illness prior to study</th>
<th>Serum antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia, chron. lymphocyt.</td>
<td>73</td>
<td>2 yr.</td>
<td>1:1024</td>
</tr>
<tr>
<td>Leukemia, chron. lymphocyt.</td>
<td>54</td>
<td>1 1/2 yr.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Leukemia, chron. lymphocyt.</td>
<td>58</td>
<td>3 yr.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Leukemia, chron. lymphocyt.</td>
<td>77</td>
<td>1 1/2 yr.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Leukemia, chron. lymphocyt.</td>
<td>71</td>
<td>16 mo.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Leukemia, chron. lymphocyt.</td>
<td>48</td>
<td>18 mo.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Leukemia, chron. lymphocyt.</td>
<td>70</td>
<td>8 mo.</td>
<td>1:2048</td>
</tr>
<tr>
<td>Leukemia, monocyt.</td>
<td>41</td>
<td>3 mo.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Leukemia, monocyt.</td>
<td>64</td>
<td>4 mo.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Leukemia, monocyt.</td>
<td>54</td>
<td>2 mo.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Leukemia, monocyt.</td>
<td>68</td>
<td>2 mo.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Leukemia, monocyt.</td>
<td>46</td>
<td>4 mo.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Leukemia, chronic granulocyt.</td>
<td>56</td>
<td>4 mo.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Leukemia, chronic granulocyt.</td>
<td>68</td>
<td>1 yr.</td>
<td>1:1024</td>
</tr>
<tr>
<td>Leukemia, subacute granulocyt.</td>
<td>41</td>
<td>1 yr.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Hodgkin's disease</td>
<td>81</td>
<td>2 1/2 yr.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Lymphosarcoma</td>
<td>60</td>
<td>2 yr.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Lymphosarcoma</td>
<td>16</td>
<td>6 mo.</td>
<td>1:128</td>
</tr>
<tr>
<td>Lymphosarcoma</td>
<td>63</td>
<td>2 mo.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Lymphoma, type undetermined</td>
<td>55</td>
<td>1 1/2 yr.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Lymphoma, type undetermined</td>
<td>67</td>
<td>14 mo.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Lymphoma, lymphocyt.</td>
<td>72</td>
<td>2 yr.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Lymphoma, lymphocyt.</td>
<td>77</td>
<td>8 mo.</td>
<td>1:4096</td>
</tr>
<tr>
<td>Malignant reticuloendotheliosis</td>
<td>13</td>
<td>4 mo.</td>
<td>1:128</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>68</td>
<td>3 mo.</td>
<td>1:1024</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>62</td>
<td>7 mo.</td>
<td>1:4096</td>
</tr>
</tbody>
</table>

*Patient had oral moniliasis.
†Patient presented as a refractory megaloblastic anemia. Eventually evolved into monocytic leukemia; terminally disseminated moniliasis.

The antigenic properties of Candida albicans appear to lie in the polysaccharide capsule which partially shares a common antigen with the capsule of Saccharomyces cerevisiae and other yeastlike organisms. The one exception to this cross reactivity is Cryptococcus neoformans. The specificity of the antibody to Candida albicans as measured by this technic is unclear at the present time. A close relationship, however, between the Candida antigen and this antibody was demonstrated in three ways. The antigenic stimulation of normal rabbits with Candida albicans significantly increased the amount of circulating antibody in the sera of normal rabbits. The antibody in the serum reacting with Candida albicans was completely absorbed with the yeast form of Candida albicans. Although rice starch is a polysaccharide against which antibody may almost invariably be found in sera of adults, repeated absorptions of the serum with this material failed to cause a diminution of serum reactivity to Candida antigen. This antibody, therefore, does not react with all polysaccharides and shows considerable specificity for the Candida group. Evidence also was obtained that this antibody occurs in the gamma globulin portion of the
Table 2.—Effects of Tumor Therapy on Titer of Serum Antibody Reacting with Candida Albicans

<table>
<thead>
<tr>
<th>Disease</th>
<th>Types of therapy</th>
<th>Duration of therapy</th>
<th>Serum antibody titer Before therapy</th>
<th>Serum antibody titer After therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia, monocytic</td>
<td>Prednisone, 6-mercaptopurine</td>
<td>Intermittently for 6 months</td>
<td>1:4096</td>
<td>1:4096</td>
</tr>
<tr>
<td>Leukemia, monocytic</td>
<td>Prednisone, 6-mercaptopurine</td>
<td>8 weeks</td>
<td>1:4096</td>
<td>1:4096</td>
</tr>
<tr>
<td>Leukemia, monocytic</td>
<td>Prednisone, 6-mercaptopurine</td>
<td>Intermittently for 4 years</td>
<td>not</td>
<td>done</td>
</tr>
<tr>
<td>Lymphoma, type undetermined</td>
<td>X-ray and nitrogen mustard</td>
<td>8 months</td>
<td>1:4096</td>
<td>1:4096*</td>
</tr>
<tr>
<td>Leukemia, chronic lymphocytic</td>
<td>Prednisone, Chlorambucil</td>
<td>Intermittently for 12 months</td>
<td>1:1024</td>
<td>1:1024</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>Local x-ray, Prednisone, Chlorambucil</td>
<td>Intermittently for 12 months</td>
<td>1:4096</td>
<td>1:4096</td>
</tr>
</tbody>
</table>

*Terminal specimens.

serum although its presence in the other serum protein fractions has not been determined.

The concept of “natural” serum antibody is ill defined but implies that such antibody occurs without deliberate antigenic stimulation. These antibodies may arise through subclinical infection with an organism or in response to an infectious agent whose portal of entry was not through the usual or suspected means. “Normal” or “natural antibodies” may be produced in response to certain antigenic materials entering the body, such as food, which contain or share a common antigen. Examples of this might include erythrocyte isohemagglutinins, rice starch antibody and antibody to various yeast organisms. This is a hypothesis, however, which is based on little experimental evidence but is supported to some extent by the observations that blood groups A and B isohemagglutinins do not develop in mice when their diet and environment are devoid of the appropriate antigens. It also has been demonstrated in rabbits that injections of rice starch will produce an appreciable serum antibody response. Similar results with Candida albicans were obtained in these studies. High antibody titers on cord blood are consistent with the observations that antibody globulin is capable of crossing the placental barrier. The low serum antibody titers at 3 months of age were obtained at a time when the newborn infant could synthesize little antibody globulin. At this time the antibody, presumably transferred to the fetal circulation from the mother, has almost completely disappeared. At 4 months of age and thereafter progressively increased amounts of serum antibody were detected in the normal subjects studied. This probably is explained by the fact that increased amounts of antibody are formed throughout life following extended and repeated environmental contact with Candida albicans and/or related organisms. Some thought has been given to the consideration that the significant incidence of thrush in infancy may be related to the low serum antibody titers observed. Consistent with this concept also is the observation that almost all individuals past adole-
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Candida-albicans show significant Candida-reacting antibody in their sera. The serum antibody titers rarely were carried beyond a dilution of 1:4096. In many of the normal subjects, however, titers as high as 1:16,384 were found. Serum dilutions were not carried beyond 1:4096 in this study since it was felt that only marked reduction below this level could be of clinical importance.

The most important consideration in these studies was whether or not patients with neoplastic disease, either with or without antineoplastic therapy, were incapable of normal serum antibody production of this type. This should be reflected by a low serum antibody titer. It has been repeatedly demonstrated that patients with neoplastic processes respond poorly to active antigenic stimulation. This deficiency appears generalized1-3 and is illustrated by anergy to tuberculin, lowered incidence of positive serological tests for syphilis, reduced or absent de novo synthesis of antibody to Brucella and Salmonella and by their increased tolerance of skin homografts.4 There was no evidence from this study that the quantity of Candida-reacting serum antibody was affected by the progression of neoplastic growth. It does seem likely, however, that the severe impairment of globulin production which may develop in terminal neoplasia could result in depression of "natural antibody" synthesis. Depression of synthesis of specific gamma globulins by ACTH and adrenal cortical steroids may be accomplished in certain laboratory animals.5,6 Ionizing radiation and nitrogen mustard also have been shown to inhibit antibody formation.4 The administration of 6-mercaptopurine to rabbits seems to impair antibody synthesis to heterologous antigen.2 Although all of these modalities were used in the treatment of patients in this study, in no instance was there reduced serum antibody reactivity. This suggests that the antibody-producing mechanism of this type is extremely resistant to the adverse effects of neoplastic growth and chemotherapeutic agents.

The lack of correlation between serum antibody titer and mycotic infection is in accord with the concept that humoral antibody is not absolute evidence of immunity.7-9 It is possible, however, that in certain specific body fluids such as the cerebrospinal fluid, the antibody concentrations may be sufficiently reduced as to allow invasion of mycotic organisms. The importance of antibody in the bacterial defense mechanism suggests that antibody to monilia probably plays a similar role. However, as with bacterial infection, this is but one factor among many which operates to repel tissue invasion. It would seem that alteration of tissue defense mechanisms (i.e., leukocytes, complement, bacterial flora, etc.) rather than humoral antibody account for the increased incidence of disseminated monilial infection in patients with lymphoma and leukemia.

SUMMARY

1. The quantity and characteristics of antibody reacting with Candida albicans was determined in normal subjects and in patients with lymphomas and leukemias by the immune-adherence technic.

2. Little antibody to Candida albicans is present in infants during the first few months of neonatal life; a progressive increase in antibody occurs during
adolescence, and antibody is present in high titer in the serum of normal adults.

3. No significant decrease in antibody titer was found in the sera of patients with advanced leukemia or lymphoma, most of whom had had extensive specific therapy.

4. This antibody was characterized by lack of cross absorption with rice starch polysaccharide, complete absorption with *Candida albicans* and by increased serum reactivity following active antigenic stimulation in rabbits.

5. It is apparent from this study that production of the "natural antibody" of this type is maintained despite the progression of neoplastic disease and the use of antineoplastic agents.

6. It is probable that alteration of tissue defense mechanisms rather than humoral antibody account for the increased incidence of disseminated monilial infections in patients with lymphomas and leukemias.

**SUMMARIO IN INTERLINGUA**

1. Le quantitate e le characteristicas de anticorpore reagente con *Candida albicans* esseva determinate in subjectos normal e in patientes con lymphomas e in patientes con le un o le altere del leucemias. Le technica usate esseva illo de adherentia immunologic.

2. Pauc anticorpore a *C. albicans* es presente in infantes durante le prime menses del vita neonatal. Un augmento progressive de iste anticorpore occurre durante le adolescence. Le anticorpore es presente in alte titros in le sero de adultos normal.

3. Nulle significative reductiones del titros de anticorpore esseva trovate in le seros de patientes con formas avantiate de leucemia o de lymphoma. Le majoritate de istes habeva habite extense cursos de therapia specific.

4. Le anticorpore esseva characterisate per absentia de absorption cruciate con polysaccharido de amylo de ris, complete absorption cin *C. albicans*, e augmento del ractivitate seral post active stimulation antigenic in conilios.

5. Il es apparente ab iste studio que le production del "anticorpore natural" de iste typo es mantenite in despecto del progression del morbo neoplastic e etiam in despecto del uso de agentes antineoplastic.

6. Il es probable que un alteration del mechanismos de defensa tissutal plus tosto que le anticorpore humoral explica le augmentate incidentia de disseminate infectiones monilial in patientes con lymphomas e le leucemias.

**REFERENCES**


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