Transformation of Lymphocytes of Normal and Hospitalized Adults by *Candida albicans* Extract[1] (37691)

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Impaired *in vitro* lymphocyte tranformation in response to a variety of naturally occurring microbial antigens occurs in several diseases having known immunological defects (1–3). Impairment of candida extract (*Candida*)-induced transformation occurs in a number of immune disorders characterized by mucocutaneous candidiasis (4, 5). However, the degree of transformation of lymphocytes from normal persons as compared to hospitalized patients in general has not been adequately defined. The present study evaluates candida-induced *in vitro* transformation of lymphocytes of patients hospitalized for reasons other than recognized immunological disorders.

Methods. Patients were ill hospitalized males from the Medical and Pulmonary Services of the Nashville Veterans Administration Hospital.

Normal control subjects were adult male hospital employees with no evidence of acute illness including minor respiratory infection who were taking no medication. Lymphocytes from heparinized venous blood were partially purified by passage through a cotton wool column and cultured in tissue culture medium 199 (Bioquest) supplemented with 20% autologous plasma as previously described (6). Each culture contained approximately 1 million lymphocytes in 2 ml plasma-supplemented medium incubated at 37° in a humidified atmosphere containing 5% CO_2. Lyophilized phytohaemagglutinin (PHA) (Difco PHA-P) was reconstituted to 5 ml, then further diluted 1:40 with Hanks buffered salt solution. One-tenth of a milliliter diluted PHA (70 μg) was added per culture. *Candida albicans* extract (candida, 1:10 Hollister Stier) was dialyzed three times against 100 vol 0.9% NaCl and once against 50 vol Hanks buffered salt solution. The dialyzed extract was filtered (Millipore filter 0.45 μm). Protein content as determined by the Lowry method (7) was 110 μg/ml. Two-tenths of a milliliter (22 μg protein) was added to each culture. PHA-stimulated cultures were incubated a total of 4 days and candida-stimulated cultures a total of 6 days. Tritiated thymidine (2 μCi) (New England Nuclear, specific activity 6.7 Ci/mmole) was added to each culture 18 hr before termination to assess thymidine incorporation as an indicator of lymphocyte blastogenesis. Methods for termination of cultures and determination of tritiated-thymidine content of the trichloroacetic acid-precipitable residue have been previously described (4). Counts per minute per culture were corrected to cpm per million cells. Results of the mean cpm per million cells for duplicate cultures are recorded herein. Effects of antigen concentration and cell number were determined from studies of lymphocytes from normal subjects.

Results. Preliminary studies. Thymidine uptake indicating blastogenesis in response to PHA varied in an almost linear fashion in relation to the number of cells per culture between 250,000 and 1,500,000 cells; and candida-induced thymidine uptake was also related to number of cells between 500,000 and 1,500,000 cells per culture as indicated in Fig. 1. Accordingly, the data has been expressed as cpm per million cells. Mean cell

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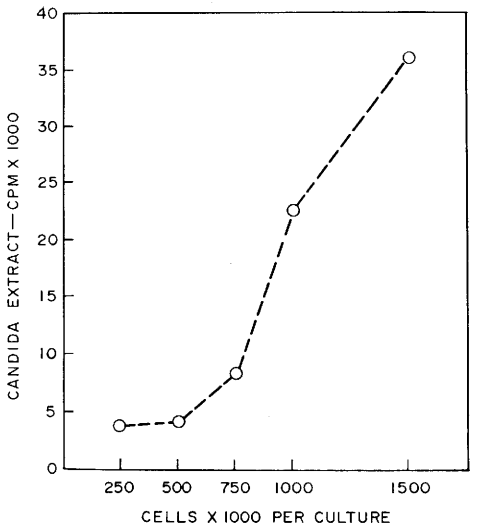

FIG. 1. Relationship between candida-induced lymphocyte transformation of a representative individual (vertical axis) and number of lymphocyte cells per culture (horizontal axis).

number per culture of 60 hospitalized subjects was 997,000 cells (± 248,000 SD) of normal controls, 1,015,000 cells (± 184,000 SD).

Increasing antigen concentration up to the maximum available was correlated with increasing thymidine uptake, indicating the lack of lymphocyte toxicity of the dialyzed antigen preparation.

Comparison of hospitalized patients and normal subjects. The mean and median values for control, PHA- and candida-stimulated lymphocyte cultures are depicted in Table I. Individual thymidine uptake values are graphically recorded in Fig. 2. No differences in PHA reactivity of lymphocytes were detected between the hospitalized and normal groups. Candida-induced lymphocyte reactivity, in contrast, differed considerably between patient and control groups, with a particularly wide range of reactivity in hospitalized subjects. Comparison of the candida-induced values for the two groups by a nonparametric method, Wilcoxon's two-sample test (8), indicated a statistically significant difference between normal and hospitalized subjects ($Z = 2.32$, $p = 0.02$).

A commonly used convention for expressing lymphocyte transformation results is the stimulation index (S/C), the ratio between the tritiated thymidine uptake of a stimulated (S) culture and a simultaneous unstimulated control (C) culture. Figure 3 records the results of converting data to the stimulation index. This ratio, though yielding wider variation, is included because of its widespread usage in evaluation of an *in vitro* immunological function. No normal subject had a candida-induced thymidine uptake of less than 5000 cpm or S/C less than 4. Consequently, those values were arbitrarily chosen as the lower limits of "normal" in the present study.

A total of 27 (45%) hospitalized subjects had either candida-stimulated cpm less than 5,000 (20 subjects), a stimulation index less than 4 (20 subjects), or both (13 subjects, 22%). Only two of these 13 subjects who were particularly hyporesponsive to candida had PHA stimulation *less* than 70,000 cpm (the lowest value in the normal group). Conversely, 16 (27%) hospitalized patients with PHA-induced transformation greater than 70,000 cpm had candida-induced transformation less than 5,000 cpm.

Discussion. Both PHA- and antigen-induced lymphocyte transformation presumably are functions of thymus-derived (T) lymphocytes (9). It, therefore, is reasonable to question whether there is really any need to evaluate *in vitro* sensitivity of lymphocytes to a

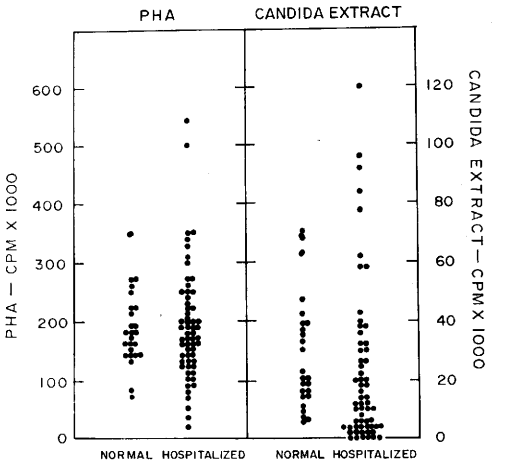

FIG. 2. PHA- and candida-induced lymphocyte transformation of normal and hospitalized subjects compared. Values are expressed as cpm per million cells.

TABLE I. *In Vitro* Lymphocyte Blastogenesis of Normal Persons and Hospitalized Patients.

Study group	No.	Tritiated-thymidine uptake (cpm/1 million cells)		
		Unstimulated mean (median)	PHA mean (median)	Candida mean (median)
Normal	25	2640 (1190)	185,700 (182,000)	28,000 (22,300)
Hospitalized	60	3040 (1142)	191,300 (172,000)	22,700 (12,200)

ubiquitous antigen. Marked impairment or absence of PHA-induced transformation is presumptive evidence of defective T lymphocyte function (9). Quantitative assays of PHA-induced transformation (10, 11) have been more discriminating in identifying suboptimal lymphocyte reactivity than routine PHA methods, but are not widely used because of their relative complexity.

Testing of antigen-induced transformation has been extensively utilized in an effort to define somewhat more physiological equivalents of lymphocyte function. Since delayed-type skin testing reactivity to *Candida albicans* extract occurs in up to 95% of normal adults (12), this antigen has been enlisted to assess antigen-mediated transformation. The Hollister-Stier antigen has been widely utilized *in vitro*. It produced significant lymphocyte transformation in 21 of 25 normal subjects evaluated by a slightly different technique (13).

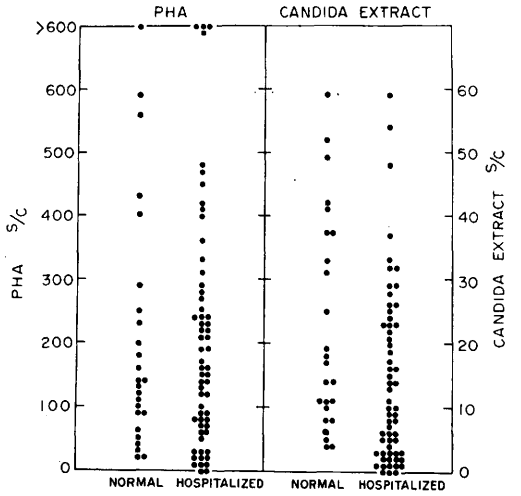

FIG. 3. PHA- and candida-induced lymphocyte transformation compared. Values are expressed as the stimulation index (S/C).

All normals in the present study had candida-reactive lymphocytes ($> 5{,}000$ cpm, $S/C > 4$). This apparent universal reactivity may, in part, have resulted from careful exclusion of those with even minor infections, since viral infections may be associated with impairment of lymphocyte transformation (14). Nonetheless, the degree of reactivity to candida extract verifies the usefulness of this natural antigen for assessing cellular immunity.

Lack of agreement between PHA- and candida-induced transformation of lymphocytes from normal subjects and particularly from hospitalized patients was demonstrated in the present study. Sixteen hospitalized subjects with PHA-induced transformation of *greater* than 70,000 cpm had candida-induced transformation of less than 5,000 cpm. Only two hospitalized subjects having hypoactive candida-induced transformation indicated by $< 5{,}000$ cpm and $S/C < 4$ had PHA-induced transformation of *less* than 70,000 cpm. Therefore, the candida-induced transformation test delineates individuals having suboptimal lymphocyte function not identifiable by routine PHA-induced transformation.

Definition of "abnormal" cellular immunity *in vitro* or especially *in vivo* is still problematic. Low stimulation indices in hospitalized subjects in part resulted from high control counts which were encountered more often in that group. Low stimulation indices on this basis may not necessarily indicate impairment of immunity at all, but could represent *in vivo* stimulation of lymphocytes thus blunting the apparent efficacy of *in vitro* stimulation. *In vivo* lymphocyte stimulation

with resulting blastogenesis of lymphocytes not further stimulated *in vitro* has been demonstrated in renal allograft recipients with active rejection (13) and blood transfusion recipients (14), and might also occur due to a variety of infections or acquired exogenous antigens, including sensitizing drugs (15). Counts of less than 5,000 cpm from candida stimulation of lymphocytes of hospitalized patients, though lower than any obtained from normal subjects, cannot be interpreted as definitive evidence of impaired antigen-induced transformation, since they were often associated with stimulation indices greater than four. The occurrence of both low unstimulated and low candida-induced tritiated thymidine uptake and a low stimulation index observed in 13 (22%) hospitalized patients provided the most convincing evidence of impaired candida-induced lymphocyte transformation. These hyporeactive patients had a variety of seemingly unrelated disorders—COPD (3), hepatitis (2), pneumonia (2), carcinoma (2), uremia, Hodgkins disease, aplastic anemia, and myocardial infarction. However, they shared a measurable diminution of one parameter of immune responsiveness—peripheral lymphocyte reactivity to a complex natural antigen. The relationship of this common occurrence to the pathogenesis of the underlying disease or the development of microbial superinfection is speculative. Perhaps this presumably acquired defect in cellular immunity to candida antigen predisposes to candida infections in hospitalized patients in concert with other known predisposing forces.

Although the cause of hyporeactive lymphocyte transformation to candida in such patients is conjectural, the phenomenon appears to be a common occurrence which may have significant implications. It is imperative to examine a matched control group of comparably ill hospitalized patients before concluding that any subset of hospitalized patients has a unique impairment of candida-induced lymphocyte transformation.

Summary. Candida extract caused significant tritiated-thymidine uptake ($> 5{,}000$ cpm, $S/C > 4$) in lymphocyte cultures from all normal adults tested. In contrast, in 22% of hospitalized patients, the antigen produced transformation less than any normal subject, and $S/C < 4$. The difference in candida-induced transformation between normal and hospitalized subjects was statistically significant, while no significant difference in conventional PHA stimulation could be demonstrated.

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