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Cell-Mediated Immunity in Diabetes Mellitus

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Cell-mediated immunity was evaluated in patients with diabetes mellitus by delayed hypersensitivity skin tests and in vitro lymphocyte transformations. Only 44% of diabetic patients had skin test reactivity to *Candida* antigen, compared with 88% of normal controls ($P < 0.001$). Insulin-dependent diabetic (IDD) patients had abnormally low lymphocyte transformation responses to phytohemagglutinin, concanavalin A, and streptokinase-streptodornase ($P < 0.05$). This defect was not corrected by culturing the cells in nondiabetic plasma. IDD patients with persistent hyperglycemia (fasting serum glucose level, >200 mg/dl) had lower levels of transformation than did IDD patients with fasting serum glucose levels less than 150 mg/dl. Lymphocytes from two IDD patients with poor lymphocyte transformation responses had marked improvement in response to phytohemagglutinin when the lymphocytes were cultured after a preincubation period designed to deplete cultures of suppressor activity. Seven IDD patients were studied serially over 12 months. Lymphocyte transformation responses in four of these patients improved coincidentally with a change in the level of fasting hyperglycemia from >200 to <150 mg/dl. The other three IDD patients with consistent fasting serum glucose levels of >200 mg/dl had poor lymphocyte transformation responses. Diabetic patients have demonstrable defects in lymphocyte function which improved in a small number of patients with reduction in the level of fasting hyperglycemia.

Patients with diabetes mellitus have an increased incidence of infections caused by bacteria, virus, and fungi (19, 20). Immune deficiencies are often invoked to explain their increased incidence of infections and morbid complications. Although humoral immunity appears to be normal in most diabetic patients (8), several types of functional abnormalities have been demonstrated in polymorphonuclear leukocytes, particularly when the patients are in ketoacidosis (2, 4, 11, 15).

Studies of cell-mediated immunity of the delayed type (CMI) have shown conflicting data in patients with diabetes mellitus (3, 5, 9, 16). CMI appears to be important in host defenses against certain infections, especially those caused by fungi and mycobacteria (6, 7, 12, 17).

The purpose of this study was to investigate CMI in diabetic patients by utilizing delayed hypersensitivity skin tests, in vitro lymphocyte transformations, and an assay of lymphocyte suppressor activity.

MATERIALS AND METHODS

Patients. Fifty diabetics from an urban county hospital were studied. Forty-four were selected randomly during clinic visits, and six patients were studied while they were hospitalized for ketoacidosis. Seven patients were studied from three to eight times during 1 year. Informed written consent was obtained before their participation in this study. The population consisted of 25 males and 25 females, with a mean age of 50 years (range, 19 to 82). Thirty-eight were insulin-dependent diabetics (IDD), and 12 were non-insulin-dependent diabetics (NIDD) receiving oral hypoglycemic agents in different combinations. Many of our patients were noncompliant in attending clinic, taking medication, or following dietary restrictions. For these reasons, only 26% of the population had fasting serum glucose (FSG) levels of <150 mg/dl, and 45% had FSG levels of >200 mg/dl when they were studied initially.

Fifty-six controls with a mean age of 49 years (range, 18 to 83) and equal sex distribution were either patients from nondiabetic clinics or healthy medical personnel. The outpatient controls had a variety of underlying diseases, such as hypertension, arteriosclerosis, congestive heart failure, and obesity. The control patients were screened so that none had any endocrinological or immunological abnormalities or serious recurrent infections.

Skin tests. Antigens used were tuberculoprotein (5 tuberculin units per 0.1 ml; Parke, Davis & Co., Detroit, Mich.), mumps skin test antigen (undiluted; Eli Lilly & Co., Indianapolis, Ind.), *Candida* (1:200 dilution; Hollister-Stier Laboratory, Dallas, Tex.), and streptokinase-streptodornase (SK-SD; 40 U of SK per 0.1 ml; American Cyanamid Co., Lederle Laboratories Div., Pearl River, N.Y.). The same batch of antigens

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was used throughout the study. Antigens were injected intradermally in 0.1-ml volumes on volar surfaces of forearms. Induration was measured at 48 to 72 h after antigen injection and recorded as positive if induration was greater than 5 mm with mumps, *Candida*, or SK-SD antigen and greater than 10 mm with tuberculoprotein antigen.

Lymphocyte transformation. Patients were studied from one to eight times over 12 months, with only one observation made in most of the patients. Heparinized peripheral blood was sedimented for 2 h at 37°C. Leukocyte-rich plasma was centrifuged, and the cells were washed with Hanks balanced salt solution (Grand Island Biological Co., Grand Island, N.Y.). Cells were suspended in RPMI 1640 tissue culture medium (Grand Island Biological Co.) and counted with a hemocytometer.

Cultures were established in triplicate in microtiter plates (Falcon Plastics, Oxnard, Calif.) containing 80% RPMI 1640 medium and 20% autologous plasma. In some experiments, pooled plasma from normal controls was substituted for autologous plasma. A total of 10^5 lymphocytes were cultured with 7.5 and 0.75 μg of phytohemagglutinin (PHA; Difco Laboratories, Detroit, Mich.) per ml, 5 μg of concanavalin A (ConA; Calbiochem, La Jolla, Calif.) per ml, SK-SD (20 U of SK per ml), or medium alone. Antigens with chemical preservatives were dialyzed against 0.85% sodium chloride for 5 days before use.

Cultures were incubated at 37°C in a 5% CO_2 humidified atmosphere for 72 to 120 h in different experiments and then pulse-labeled with 1 μCi of $[^3H]$-thymidine (specific activity, 20 μCi/mmol; New England Nuclear Corp., Boston, Mass.) for 4 h. Cultures were harvested onto glass-fiber filter papers (Reeve Angel, Clifton, N.J.) with a multiple-sample precipitator (Otto-Hiller, Madison, Wis.). Filter papers were each washed 10 times with 0.85% NaCl, 10 times with 5% trichloroacetic acid, and then 10 times with absolute methanol. After drying, filter papers were placed in a toluene-based solution (42 ml of Liquofluor in 1 liter of toluene; New England Nuclear Corp.) and counted for 1 min on a Beckman scintillation counter. Means of triplicate cultures were calculated and expressed as a ratio of mean counts per minute of the stimulated versus the unstimulated cultures to derive a stimulation index (SI).

Suppressor activity in lymphocyte cultures was assayed by the method of Stobo et al. (18), with an initial 7-day preincubation without antigen at 37°C. Lymphocyte cultures were then incubated for 5 days with PHA and fresh medium and processed as described above. Lymphocyte cultures of 25 normal controls and 15 diabetic patients were studied for suppressor activity. A percentage of suppressor activity (suppressor index) was derived by dividing the SIs obtained with the suppressor assay cultures by the PHA responses in conventional cultures and multiplying by 100.

Data were analyzed by two-tailed Student's t test.

RESULTS

Skin tests. Percentages of positive skin tests in 45 outpatient diabetics and 50 controls are shown in Table 1. There were no differences between the NIDD and the IDD, so they were combined. Of the controls, 88% exhibited delayed skin test hypersensitivity to *Candida* antigen, but only 44% of the diabetic patients had positive tests to this antigen. This difference is statistically significant ($P < 0.001$). No significant differences were noted with SK-SD, mumps, or tuberculoprotein antigens, although the percentages of positive skin tests with SK-SD and mumps antigens were lower in diabetics.

Lymphocyte transformations. Lymphocyte transformation responses of 50 diabetic patients were compared with those of 56 controls (Table 2). Lymphocytes of 12 NIDD had lower mean SIs to nonspecific mitogens (PHA, SI = 58; ConA, SI = 46) than did controls (PHA, SI = 75; ConA, SI = 60), but these differences were

TABLE 1. *Frequency of positive skin tests*

Patients	No. studied	Antigens (% positive)			
		Candida	SK-SD	Mumps	Tuberculoprotein
Normal controls	50	88	84	64	8
Diabetic	45	44[a]	73	53	13

[a] $P < 0.001$ when compared with control values.

TABLE 2. *Lymphocyte transformations*

Patients	No. studied	Antigen[a]			No antigen (cpm)
		PHA[b]	ConA	SK-SD	
Controls	56	75 ± 34	60 ± 44	18 ± 22	405
NIDD	12	58 ± 39	46 ± 37	7 ± 9[c]	403
IDD	38	37 ± 20[c]	30 ± 29[c]	5 ± 6[c]	446

[a] Mean SI ± one standard deviation.
[b] Higher response to one of two concentrations (0.75 and 7.5 μg/ml).
[c] $P < 0.05$ when compared with controls.

not statistically significant. A significant reduction in SI occurred with the specific antigen SK-SD in the NIDD groups ($P < 0.05$). In contrast, lymphocytes from the 38 IDD patients had very low mean indexes of transformation to PHA (SI = 37) and ConA (SI = 30), as well as to SK-SD (SI = 5). All these differences were statistically significant when compared with control values ($P < 0.05$). Transformations of unstimulated cultures without antigens were similar in all three groups, with mean counts per minute between 403 and 446.

A correlation was also noted in the IDD group between the SIs to PHA and the level of hyperglycemia. Mean SI with PHA was 27 in the IDD group with FSG levels of >200 mg/dl and 51 in the IDD patients with FSG levels of <200 mg/dl. Both of these IDD groups had significantly lower mean SIs than did nondiabetic controls (SI = 75 to PHA; $P < 0.05$). The mean SI with PHA of the six patients in ketoacidosis (22 ± 16) was no different than of those with FSG levels of >200 mg/dl (27 ± 21).

Suppressor activity in lymphocyte cultures. An assay of suppressor activity was performed on lymphocyte cultures from 15 IDD patients and 25 controls. No suppressor activity was seen in any control patients in this assay (suppressor indexes, 50 to 100%), except for one whose suppressor index was 108% (Fig. 1). Ten cultures for the IDD patients had a range of indexes similar to those of the controls, implying a lack of significant suppressor activity. Three of the 15 IDD patients had lymphocytes with some suppressor activity manifested by suppressor indexes between 110 and 144%. Two of the IDD patients (designated A and B) exhibited striking improvement of PHA response into the normal range after preincubation, with SIs increasing from 25 to 75 and from 30 to 83, respectively. Thus, patients A and B showed suppressor indexes of 300 and 281%, respectively (Fig. 1). Patients A and B consistently had FSG levels of >200 mg/dl, and both had episodes of keotacidosis in the month before the suppressor tests.

Effect of homologous plasma. To investigate whether the reduction in lymphocyte transformations to PHA in the IDD group was due to inhibitory factors contained in their plasma, 15 diabetics were restudied with lymphocyte transformations to PHA. Their washed lymphocytes were stimulated with 0.75 μg of PHA per ml in either autologous or normal pooled homologous plasma. The SIs ranged from 10 to 84 in autologous plasma and from 10 to 78 in homologous plasma. No enhancement of SIs was seen after substitution of normal plasma for IDD plasma. These data indicate that no plasma inhibitors to

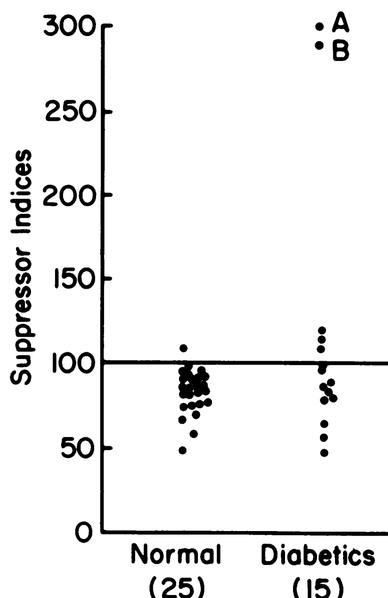

FIG. 1. *T-cell suppressor assay. Two IDD patients (A and B) with persistent hyperglycemia and a recent episode of ketoacidosis had increases in the level of response to PHA into the normal range after preincubation of lymphocyte cultures, a procedure designed to deplete the system of suppressor cells (18).*

lymphocyte transformations were found in these diabetic patients.

Longitudinal studies. Lymphocytes of seven IDD patients were studied at least three times with PHA mitogen over a 12-month period (Fig. 2). Four of these IDD patients had improved diabetic control as seen by reductions in their levels of hyperglycemia (FSG levels were reduced from >200 to <150 mg/dl). Two of these patients (no. 1 and 2) were started on insulin at the onset of this study. A third patient (no. 3) was hospitalized in the middle of the year for control of her diabetes and subsequently had increases in lymphocyte responses to PHA (SIs increased from 50 to 100). Patient no. 4 had been on insulin for 1 year, but her FSG levels were >200 mg/dl. Further regulation of her insulin dosage reduced her FSG levels to <150 mg/dl, and her SIs to PHA increased from 34 to 54. Three other patients (no. 5, 6, and 7) had consistently elevated FSG levels (>200 mg/dl) or repeated episodes of keotacidosis (no. 5 and 7). Their SIs showed abnormally low values at the termination of this study. These observations are limited, but indicate an area for future study.

DISCUSSION

Patients with diabetes mellitus appear to have an increased incidence of infections with a wide

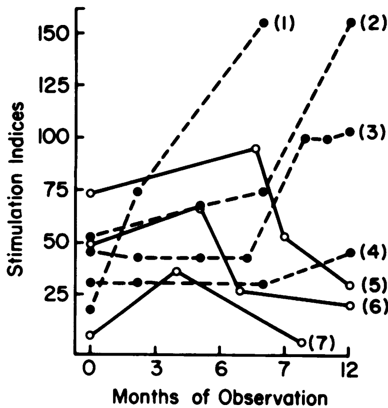

FIG. 2. *Serial SIs to PHA. Improvement in lymphocyte transformation responses to PHA was noted in four patients in whom better control of diabetes was established. Three patients consistently had levels of >200 mg/dl and had poor lymphocyte transformation responses to PHA. Symbols:* ●, *reduction in hyperglycemia to FSG levels of <150 mg/dl (no. 1, 2, 3, and 4);* ○, *persistent hyperglycemia with FSG levels of >200 mg/dl (no. 5, 6, and 7).*

variety of pathogens (19, 20). The spectrum of these organisms suggests that multiple defects occur in several areas of their immune system. Defects have been found in acute inflammatory responses of diabetic patients in ketoacidosis as measured by the skin window technique (15). In vitro function studies of polymorphonuclear leukocytes have shown frequent abnormalities in chemotaxis, phagocytosis, and intracellular killing of bacteria, especially in the poorly controlled IDD (2, 4, 11).

Whereas granulocytic leukocytes are important in defending against bacterial infections, CMI is thought to play a dominant role in protecting against fungal infections (6, 7, 12, 17).

Conflicting data have been reported on the status of CMI in diabetics. In 1970 Brody and Merlie (3) reported decreased lymphocyte transformation in six diabetics, whereas Ragab et al. (16) found normal values in 23 diabetic patients. More recent studies from Delespesse et al. (5) and MacCuish et al. (9) have found diminished lymphocyte response to PHA in poorly controlled diabetic patients with normal percentages of T cells.

This study was initiated to explain these apparently conflicting results by studying a diabetic population with both delayed hypersensitivity skin tests and lymphocyte transformation responses. A significant reduction in the number of diabetic patients responding to *Candida* skin test antigen was found. The skin test responses to mumps and SK-SD antigens, although lower in diabetics than in controls, were not statistically different. These results seem to indicate a restricted defect in CMI in *Candida,* an organism which frequently infects diabetics (19, 20). There does not appear to be a global decrease in delayed hypersensitivity which would be manifest by total skin test anergy.

IDD patients had depressed lymphocyte transformation responses to PHA, ConA, and SK-SD. This defect was especially pronounced in patients with hyperglycemia (FSG levels of >200 mg/dl) or in ketoacidosis. Lymphocytes of NIDD patients responded normally to the mitogens PHA and ConA but had decreased responses to the specific antigen SK-SD. These in vitro lymphocyte defects were not due to plasma factors, as has been shown with washed lymphocytes of patients who had liver disease (14), renal failure (13), and sarcoid (10). These mitogenic abnormalities were not corrected by culturing lymphocytes in pooled human plasma.

Fifteen diabetics were studied with a prolonged culture technique to demonstrate suppressor activity, as described by Stobo et al. in disseminated fungal infections (18). Lymphocytes from two of these diabetic patients had a marked improvement to normal SIs for PHA. One explanation for this effect is that these diabetics had excessive suppressor activity to account for their low responses to PHA noted in conventional lymphocyte cultures. Neither patient has disseminated fungal or viral disease which has been associated with increased suppressor activity (18). Interestingly, both patients had episodes of ketoacidosis in the month before these tests. Further studies were needed to determine whether recent episodes of keotacidosis are frequently associated with increased suppressor activity.

Lymphocytes from seven IDD patients were also studied from three to eight times over a year. Four of these patients had their diabetes brought under better control during the study period, as shown by significant reductions in levels of their FSGs. Three patients had marked improvement in their lymphocyte functions, as measured by increased SIs to PHA. One patient had slight improvement. Uncontrolled diabetes was seen in three other patients with frequent episodes of ketoacidosis or persistent hyperglycemia (FSG levels of >200 mg/dl). Lymphocytes from these latter three patients had abnormally low SIs to PHA. Although the numbers of patients studied were small, these data suggest that better diabetic control may enable the lymphocytes to respond normally to PHA.

Our study supports the observation that diabetic lymphocytes function abnormally (3, 5, 9). Furthermore, we have shown that, although demonstrating normal skin test response to bac-

terial and viral antigens, diabetics had significant reductions in response to a fungal skin test antigen. These patients with negative *Candida* skin tests did not have widespread disease and antigen overload such as is seen in chronic mucocutaneous candidiasis (7). Conflicting data reported in earlier studies can probably be explained best by the state of diabetic control in these study populations. These abnormal lymphocyte transformations may revert to normal responses with reduction of the hyperglycemia. We have no data as to whether the incidence of infections correlates with these lymphocyte studies. As immunological tools become more sophisticated, the basic derangement of lymphocyte function may be explained. Abnormalities may exist in membrane receptors for mitogens in these cells, as has been noted in diabetics for insulin (1), or they may reflect intracellular defects in metabolism. We have not investigated the role of the macrophage, and it is possible that abnormal macrophage function could explain in vivo and in vitro defects of CMI. These studies also may have therapeutic implications for diabetic patients with disseminated or deep-seated fungal disease. Along with better diabetic control and antibiotics, immunological agents such as transfer factor or immunoadjuvants may have a role in bolstering or augmenting CMI.

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LITERATURE CITED

1. **Archer, J. A., P. Gorden, and J. Roth.** 1975. Defect in insulin binding to receptors in obese man. J. Clin. Invest. **55**:166–174.
2. **Bagdade, J. D., R. K. Root, and R. J. Bulger.** 1974. Impaired leukocyte function in patients with poorly controlled diabetes. Diabetes **23**:9–15.
3. **Brody, J. I., and K. Merlie.** 1970. Metabolic and biosynthetic features of lymphocytes form patients with diabetes mellitus: similarities to lymphocytes in chronic lymphocytic leukemia. Br. J. Haematol. **19**:193–201.
4. **Bybee, J. D., and D. E. Rogers.** 1964. The phagocytic activity of polymorphonuclear leukocytes obtained from patients with diabetes mellitus. J. Lab. Clin. Med. **64**:1–13.
5. **Delespesse, G., J. Duchateau, P. A. Bastenic, et al.** 1974. Cell-mediated immunity in diabetes mellitus. Clin. Exp. Immunol. **18**:461–467.
6. **Hart, P. D., E. Russell, and J. S. Remington.** 1969. The compromised host and infection. II. Deep fungal infection. J. Infect. Dis. **120**:169–191.
7. **Kirkpatrick, C. H., R. R. Rich, and J. E. Bennett.** 1971. Chronic mucocutaneous candidiasis: model building of cellular immunity. Ann. Intern. Med. **74**:955–978.
8. **Lipscomb, H., H. G. Dobson, and J. A. Greene.** 1959. Infection in the diabetic. South. Med. J. **52**:16–23.
9. **MacCuish, A. C., S. J. Urbanish, C. T. Campbell, et al.** 1974. Phytohemagglutinin transformation and circulating lymphocyte subpopulations in insulin-dependent diabetic patients. Diabetes **23**:708–712.
10. **Mangi, R. J., L. M. Dwyer, and F. S. Kantor.** 1974. The effect of plasma upon lymphocyte response in vitro: demonstration of a humoral inhibitor in patients with sarcoidosis. Clin. Exp. Immunol. **18**:519–528.
11. **Mowat, A. G., and J. Baum.** 1971. Chemotaxis of polymorphonuclear leukocytes from patients with diabetes mellitus. N. Engl. J. Med. **284**:621–627.
12. **Nelson, N. A., H. L. Goodman, and H. L. Oster.** 1957. The association of histoplasmosis and lymphoma. Am. J. Med. Sci. **232**:56–65.
13. **Newberry, W. M., and J. P. Sanford.** 1971. Defective cellular immunity in renal failure: depression of reactivity of lymphocytes to PHA by renal failure serum. J. Clin. Invest. **50**:1262–1271.
14. **Newberry, W. M., J. W. Shorey, J. P. Sanford, and B. Coombes.** 1973. Depression of lymphocyte reactivity to phytohemagglutinin in serum from patients with liver disease. Cell. Immunol. **6**:87–97.
15. **Perillie, P. E., J. P. Nolan, and S. C. Finch.** 1962. Studies of the resistance to infection in diabetes mellitus: local exudative cellular response. J. Lab. Clin. Med. **59**:1008–1015.
16. **Ragab, A. H., B. Hazlett, and D. H. Cowan.** 1972. Response of peripheral blood lymphocytes from patients with diabetes mellitus to phytohemagglutinin and Candida albicans antigens. Diabetes **21**:906–907.
17. **Rosen, F. S.** 1968. The lymphocyte and the thymus gland—congenital and hereditary abnormalities. N. Engl. J. Med. **279**:643–648.
18. **Stobo, J. D., S. Paul, R. E. VanScoy, and P. E. Hermans.** 1976. Suppressor thymus-derived lymphocytes in fungal infection. J. Clin. Invest. **57**:319–328.
19. **Thornton, G. F.** 1971. Infections and diabetes. Med. Clin. North Am. **55**:931–938.
20. **Younger, D.** 1965. Infections in diabetes. Med. Clin. North Am. **49**:1005–1013.