Impaired Granulocyte Adherence

A Reversible Defect in Host Defense in Patients with Poorly Controlled Diabetes

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SUMMARY

When the ability of granulocytes from 10 poorly controlled diabetic patients with fasting hyperglycemia and no evidence of ketoacidosis (mean fasting glucose 293 ± 20 mg. per 100 ml.; mean ± S.E.M.) to adhere to a nylon fiber column was assessed, the number of adherent granulocytes from whole blood was only 53 ± 6 per cent of the values observed in controls. After antidiabetic treatment for one to two weeks and lowering of fasting glucose levels (mean 198 ± 29 mg. per 100 ml.), adherence improved significantly ($p < 0.01$) in the diabetics; however, their values were still subnormal (diabetic 74 per cent ± 8 of control; $p < 0.02$). Adherence values before and after treatment correlated with the fasting glucose level ($r = 0.88$, $p < 0.001$).

These findings suggest that, in addition to previously reported abnormalities in migration and the ingestion and killing of bacteria, granulocyte adherence may also be impaired in poorly controlled diabetic patients. This functional abnormality correlates directly with the fasting glucose and is reversed by insulin treatment. A defect of this type may compromise the normal inflammatory response in some diabetics and impair their capacity to resist infection. DIABETES 27:677-81, June, 1978.

INTRODUCTION

Before the availability of insulin and antimicrobial treatment, infection was the most frequent cause of death in the diabetic. Even with insulin treatment, infection continues to be a common cause for instability and precipitation of ketoacidosis. These observations have suggested that the diabetic patient is inherently more susceptible to infection.

Past investigations in well-controlled diabetics have failed to demonstrate any consistent abnormality in host defense that might predispose to infection. Such observations are consistent with clinical experience, which has suggested that when the clinical state of insulin deficiency is adequately treated the capacity of the diabetic to resist infection is normal. More recent studies, however, employing advanced methods have demonstrated abnormalities in granulocyte chemotaxis,[1-3] phagocytosis,[4,5] and microbicidal function[6,7] in poorly controlled diabetic patients that were reversed with more intensive insulin treatment and improved control.

A number of techniques have been used in the past to study granulocyte adherence,[8] an important early event in the normal inflammatory response. Earlier workers have suggested that this important step in host defense might be impaired in some diabetic patients[9] and that this abnormality might be related to hyperglycemia. Therefore the following studies were undertaken to determine how granulocyte adherence is influenced by hyperglycemia and clinical control of the diabetic state.

MATERIAL AND METHODS

Subjects Studied

Heparinized blood samples were obtained from 10 patients at 8:00 a.m. on the days of study after an overnight fast during a period of poor control and after 7 to 10 days of more intensive antidiabetic treatment with insulin. Eight of these patients were insulin dependent when first studied. None had evidence of renal insufficiency, infection, or ketoacidosis or was taking medications known to alter leukocyte adher-

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ence. On the days that diabetic patients were studied, blood samples were obtained under identical conditions from one or more healthy sex-matched control subjects. The same subjects served as controls for the same diabetic patients studied before and after treatment. Blood glucose was measured in all diabetic and control subjects.

The Adherence Assay System

Granulocyte adherence was studied according to the method of MacGregor et al.[10] Briefly, scrubbed preweighed nylon fiber was packed with a wooden applicator into Pasteur pipets, the tips of which had been heated and drawn out to about 50 per cent of original diameter with an aperture that admitted the shaft of a 25-gauge needle. The column length of the packed fiber was 15 mm., measured from the midpoint of pipet shoulder to the top of the column. Since we also found that polymorphonuclear neutrophil leukocytes (PMNs) from females had uniformly higher adherence to the fiber column as reported by MacGregor,[10] columns containing less fiber (70 mg.) were used for the female patients and 80 mg. of fiber were employed for males. In this way, the difference in values observed when both males and females were run on 70-mg. columns was reduced, and at the same time the sensitivity of the column to small decreases in adherence was maintained. With columns containing 70 mg. of fiber, 10 normal female volunteers showed a granulocyte adherence of 58 ± 1 per cent, mean ± S.E.M.; 10 normal males demonstrated an average adherence of 67 ± 2 per cent on the 80-mg. fiber columns.

A granulocyte count was performed on each blood sample (100 white cells are differentiated for each blood smear). Then, 1-ml. aliquots of the blood specimen were added to the tops of three pipets and allowed to filter through the columns for one to five minutes at room temperature. The granulocytes in the effluent blood were counted, and the percentage of granulocyte adherence was calculated by the following equation:

$$100\% - \left(\frac{\text{PMN/ml. in effluent sample}}{\text{(PMN/ml. in original sample}} \times 100 \right) = \text{\% PMN adherence}$$

The granulocyte adherence for a given specimen was defined as the mean value for three columns tested and was expressed as a percentage of control.

Effects of Glucose and Insulin on Adherence

In order to determine the effects of circulating glucose concentrations and insulin on granulocyte adherence, the following studies were performed. Blood was drawn from eight control subjects after an overnight fast; to 10-ml. aliquots, glucose was added isosmotically in increments to achieve four different glucose concentrations in the range of those observed in diabetic patients. All tubes to which glucose had been added and a control tube to which a volume of isotonic saline equal to that added to the tubes to which glucose was added were then incubated at 37° C. for 30, 60, and 120 minutes and adherence studies performed as described above. Results were expressed as a percentage of the adherence observed in the tubes to which saline alone had been added.

In the insulin incubation studies, fasting blood samples were collected similarly from nine diabetic and eight control subjects. To 10-ml. aliquots from each patient and control subject, porcine insulin low in phenol preservative was added (1,000 μU. per milliliter) in phosphate buffer. An equal volume of buffer was added to one 10-ml. aliquot of whole blood from each subject to serve as control. Incubation then was carried out at 37° for 60 minutes, and adherence studies then performed as described above. Results were expressed as the increment above the adherence observed in the tubes to which buffer or insulin alone had been added and not incubated.

RESULTS

Before improved antidiabetic treatment, granulocyte adherence was significantly reduced ($p < 0.01$) in the diabetic patients (table 1). After treatment, adherence improved significantly ($p < 0.02$); however, the mean performance of the group remained less than in the control group. Individual adherence responses of each patient before and after treatment expressed as a percentage of control are shown in figure 1. Fasting glucose levels fell after treatment, though the majority of patients still demonstrated fasting hyperglycemia (table 1). A highly significant inverse correlation between fasting glucose and granulocyte adherence before and after treatment was present in the diabetic patients (figure 2).

Adherence increased to a similar extent in both saline-incubated and glucose-incubated samples, so no significant difference between the saline-treated and glucose-treated samples was observed at 30 minutes (figure 3). By 60 minutes, however, a tendency toward a reduction in adherence was apparent in the glucose-treated samples; at 120 minutes, a significant diminution in adherence was observed in all samples tested ($p < 0.01$, paired t-test). Thus, adherence increased with time in the saline-incubated samples, and this tendency was reduced significantly in the presence of elevated glucose concentrations at 120

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TABLE 1

Changes in granulocyte adherence and fasting glucose values in 10 diabetic patients before and after improved treatment

	Before Treatment		After Treatment	
Patient	Glucose (mg./100 ml.)	Adherence/% control	Glucose (mg./100 ml.)	Adherence/% control
1	255	81	178	91
2	268	33	299	48
3	240	53	80	76
4	368	48	372	21
5	283	69	180	91
6	340	50	210	69
7	204	76	92	95
8	345	21	256	52
9	245	61	190	96
10	386	33	118	100
Mean ± S.D.	293 ± 62	52.5 ± 20	198 ± 92	73.9 ± 26

minutes. Adherence increased in both control and diabetic granulocytes after incubation with both insulin and buffer for 60 minutes (table 2), and no significant difference was observed in the increments observed with insulin and buffer alone.

DISCUSSION

It has been believed for many years that an abnormality in host defense predisposed the diabetic patient to both bacterial and fungal infection.[11] Despite many attempts to explain the increased frequency and severity of infection in some diabetics, until recently no abnormality in either humoral or cellular components of host defense had been identified.

Stimulated by advances in immunology and organ transplantation in the past decade, new investigative techniques have been developed, and their application has provided new information about granulocyte function and metabolism in diabetes. For example, it has been shown that chemotaxis, the directed migration of leukocytes to the site of inflammation, is delayed in diabetes, and this abnormality can be reversed in vitro by the presence of insulin.[1-3] A defect in phagocytosis, which could further delay the formation of the inflammatory response and ingestion of microorganisms, also has been demonstrated in both ketotic[12] and nonketotic[4,5,13] diabetics. This disturbance in phagocytosis, as well as an impairment in granulocyte microbicidal function recently described in some poorly controlled diabetics, also have been shown to be reversed by improved diabetic control.[6,7]

While sensitive methods have been available generally to quantitate chemotaxis, phagocytosis, and the intracellular killing of ingested organisms, there has

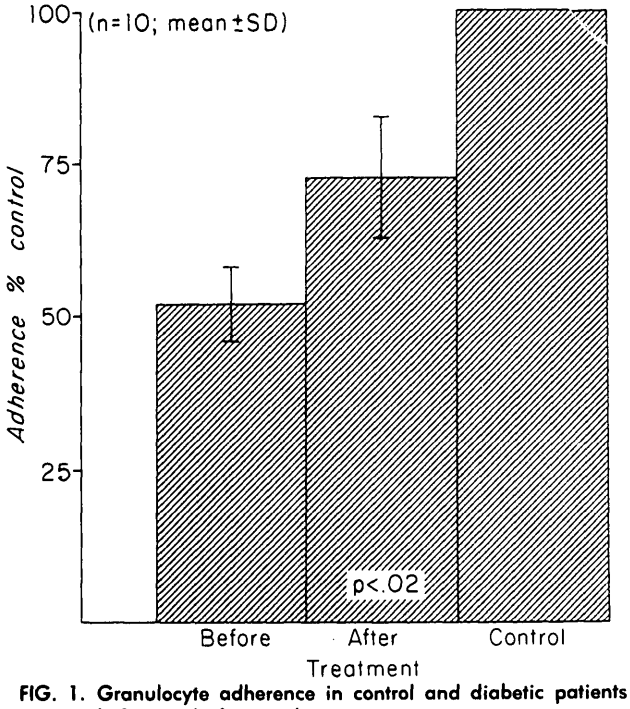

FIG. 1. Granulocyte adherence in control and diabetic patients before and after insulin treatment.

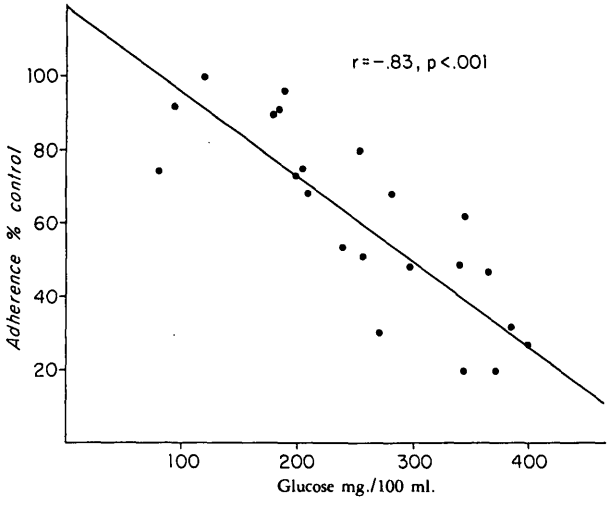

FIG. 2. Relationship between granulocyte adherence and fasting glucose concentrations in diabetic patients before and after treatment.

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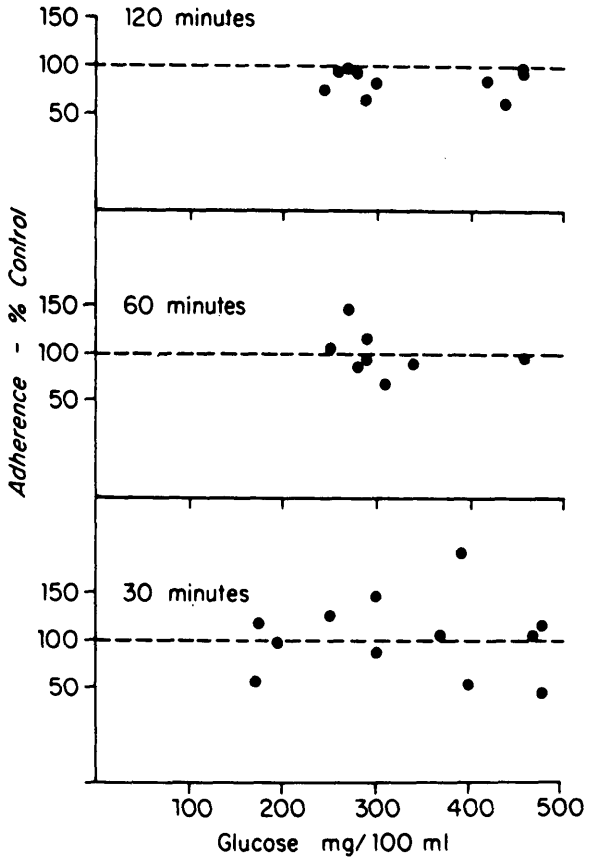

FIG. 3. Effect of increasing glucose concentrations and time on granulocyte adherence in control subjects. Results expressed as percentage of adherence in tube incubated same duration of time with saline alone.

been no means of assessing the adherence of granulocytes to vascular endothelium, the essential step in host defense that precedes diapedesis and exudate formation in extravascular tissues. The recently developed nylon fiber column technique[10] employed in this study makes it possible to indirectly estimate this important granulocyte function in vitro. We have found that when granulocyte adherence is studied in this way, the performance of PMNs from poorly controlled diabetic patients is impaired frequently, and, like the functional abnormalities shown earlier in phagocytosis and microbicidal function, this disturbance also is reversed with insulin treatment. Because of the limitations of this method, caution must be exercised in extrapolating the abnormality observed in the ability of diabetic PMNs to adhere to nylon fibers to the clinical situation of adherence to vascular endothelium. Nevertheless, the close relationship shown between the severity of the abnormality in adherence and the fasting glucose value is similar to the correlation between the degree of diabetic control and the efficiency of phagocytosis described earlier in similar poorly controlled diabetics.[4,5] It is of interest that standard techniques employed to measure chemotaxis simultaneously assess PMN adherence, since PMNs must adhere to the surface of various chamber assay systems. Our finding that adherence is defective in diabetic patients[1,2] indicates that past reports of a delay in the directed migration of PMNs in vitro may be due to the abnormality we observed in adherence. Further studies, therefore, are required with techniques that clearly dissociate the separate steps of adherence from chemotaxis in order to assess these two vital steps in host defense.

The mechanism(s) contributing to impaired adherence in these diabetic patients is unclear. There is no evidence to date to indicate that serum factors unrelated to glucose such as opsonins or complement, which are necessary for normal granulocyte phagocytic function, are altered in the diabetic; hence, it seems unlikely that a disturbance in the complement system contributed to their decrease in adherence. Our finding that increasing glucose concentrations reduce granulocyte adherence is consistent with Van Oss's earlier report.[9] These findings, together with our earlier observation that the isosmotic addition of glucose to control granulocytes also impairs their phagocytic capacity, suggest that hyperglycemia alone may induce metabolic alterations that compromise the normal functional capacity of the granulocyte.

Chemotaxis, adherence, and phagocytosis all have been shown to be impaired in diabetics, and each of these important granulocyte functions has been shown to be an energy-dependent process. Granulocytes normally derive energy almost exclusively from the metabolism of glucose.[14] The enhanced metabolic activity of stimulated PMNs is reflected by the fact that, during phagocytosis, their glycogen concentrations fall and oxygen consumption, glucose utilization, and lactate production all increase.[15] The fact that these parameters of metabolic activity all have been shown to be impaired in leukocytes from poorly controlled diabetic patients[14,15] supports the possibility that the functional disturbances reported in diabetic granulocytes may result from defective energy production.

It is known, for example, that in the presence of

TABLE 2

Increases in granulocyte adherence after incubation for 60 minutes with buffer or insulin (1,000 μU. per milliliter) above the values obtained without incubation

	Buffer	Insulin	P
Diabetic (n=6)	+61 ± 18.6	+43.7 ± 25.7	> 0.1
Control (n=8)	+22 ± 9.25	+33.3 ± 22.4	> 0.1

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insulin deficiency, PMN glycogen content[8] and the activity of glycogen synthetase are both reduced.[9] While most enzymatic steps in the glycolytic pathway are reversible, the hexokinase, phosphofructokinase, and pyruvatekinase reactions are not; of these, the latter two are closely regulated by insulin availability.[20] The combined findings in the diabetic PMN of an inhibition of the hexokinase reaction, an accumulation of fructose-6-phosphate, and a reduction of pyruvatekinase activity are consistent with blocks in the glycolytic pathway at these critical insulin-dependent steps.[17] The reversibility of these specific metabolic disturbances with insulin treatment is consistent with the hypothesis that the improvement observed in granulocyte adherence in the present study and in past clinical studies of phagocytosis and microbicidal function in similar diabetic patients may be a consequence of impaired energy production. The lack of improvement in adherence in the 60-minute incubation study with insulin suggests that more prolonged exposure to insulin might be required to improve metabolic function in the diabetic granulocyte. Similar pathways of glucose metabolism have been demonstrated in lymphocytes from patients with diabetes.[21] The recent finding that animals rendered diabetic with streptozotocin demonstrate a number of lymphocyte-mediated immunologic abnormalities[22] suggests that disorders causing delayed hypersensitivity, such as tuberculosis, may be more common in the diabetic because of impaired lymphocyte function. Despite the abnormalities in granulocyte adherence found in the participants in our study, none had clinical evidence of infection. This finding is consistent with clinical experience, which suggests that host defense mechanisms have considerable reserve and must be severely compromised before microbial invasion and dissemination actually occur.

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REFERENCES

[1]Mowat, A. G., and Baum, J.: Chemotaxis of polymorphonuclear leukocytes from patients with diabetes mellitus. N. Engl. J. Med. *284*:621-27, 1971.

[2]Hill, H. R., Sauls, H. S., Dettloff, J. L., and Quie, P. G.: Impaired leukocyte responsiveness in patients with juvenile diabetes mellitus. Clin. Immunol. Immunopathol. *2*:395-403, 1974.

[3]Miller, M. E., and Baker, L.: Leukocyte functions in juvenile diabetes mellitus: humoral and cellular aspects. J. Pediatr. *81*:979-82, 1972.

[4]Bagdade, J. D., Nielson, K. L., and Bulger, R.: Reversible abnormalities in phagocytic function in poorly controlled diabetic patients. Am. J. Med. Sci. *263*:451-56, 1972.

[5]Bagdade, J. D., Root, R. K., and Bulger, R. J.: Impaired leukocyte function in patients with poorly controlled diabetes. Diabetes *23*:9-15, 1974.

[6]Tan, J. S., Anderson, J. L., Watanakunakorn, C., and Phair, J. P.: Neutrophile dysfunction in diabetes mellitus. J. Lab. Clin. Med. *85*:26-33, 1975.

[7]Nolan, C. M., Beaty, H. N., and Bagdade, J. D.: Impaired granulocyte bactericidal function in patients with poorly controlled diabetes. Diabetes *27*:1978. In press.

[8]Penny, R., Galton, D. A. G., Scott, J. T., and Eisen, V.: Studies on neutrophile function. I. Physiological and pharmacological aspects. Br. J. Haematol. *12*:623-32, 1966.

[9]Van Oss, C. V.: Influence of glucose levels on the *in vitro* phagocytosis of bacteria by human neutrophiles. Infect. Immun. *4*:51-59, 1971.

[10]MacGregor, R. R., Spagnulo, P. J., and Lentnek, A. L.: Inhibition of granulocyte adherence by ethanol, prednisone, and aspirin, measured with an assay system. N. Engl. J. Med. *291*:642-46, 1974.

[11]Johnson, J. E.: Infection and diabetes. *In* Diabetes Mellitus: Theory and Practice. Ellenberg, M., and Rifkin, H., Eds. New York, McGraw-Hill, 1970, pp. 734-45.

[12]Bybee, J. D., and Rogers, D. E.: The phagocytic activity of polymorphonuclear leukocytes obtained from patients with diabetes mellitus. J. Lab. Clin. Med. *64*:1-13, 1964.

[13]Baciu, I., Cerevenco, V., Vitebski, V., et al.: Influenta insulinei si a glucozei asupra functiei facocitare c si mibilitatii leucocitelor. Stud. Cercet. Endocrinol. *18*:121-29, 1967.

[14]Stjernholm, R. L., Burns, C. P., and Hohnhadel, J. H.: Carbohydrate metabolism by leukocytes. Enzyme *13*:7-31, 1972.

[15]Klebhanoff, S. J.: Antimicrobial mechanisms in leukocytes. Semin. Hematol. *12*:117-42, 1975.

[16]Martin, S. P., McKinney, G. R., Green, R., and Becker, C.: The influence of glucose, fructose, and insulin on the metabolism of leukocytes of healthy and diabetic subjects. J. Clin. Invest. *32*:1171-74, 1953.

[17]Esmann, V.: The diabetic leukocyte. Enzyme *13*:32-55, 1972.

[18]Dahlquist, A., Gahrton, G., and Norden, A.: The content of total and acid-soluble glycogen in the white blood cells and its relation to the staining with the periodic acid Schiff reagents. Acta Med. Scand. *172*:31-40, 1962.

[19]Esmann, V., Hedeskov, C. J., and Rosell Perez, M.: UDP-glucose-glucosyltransferase activity of polymorphonuclear leukocytes from diabetic subjects. Diabetologia *4*:181-87, 1968.

[20]Weber, G., Lea, M. A., Fisher, E. A., and Stamm, N.: Regulatory pattern of liver carbohydrate metabolizing enzymes: insulin as inducer of key glycolytic enzymes. Enzymol. Biol. Clin. 7:11-24, 1966.

[21]Brody, J. I., and Merlie, K.: Metabolic and biosynthetic features of lymphocytes from patients with diabetes mellitus: similarities to lymphocytes in chronic lymphocytic leukemia. Br. J. Hematol. *19*:193-201, 1970.

[22]Mahmoud, A., Rodman, H., Mandel, M., and Warren, K.: Induced and spontaneous diabetes mellitus and suppression of cell-mediated immunologic responses. J. Clin. Invest. *57*:362-67, 1976.

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