Inhibition of Leukocyte Chemotaxis by Factor in Alloxan-Induced Diabetic Rat Plasma

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SUMMARY

The early local exudative cellular reaction in an inflammatory lesion was impaired in alloxan-induced diabetic rats due to reduced migration of neutrophils to the inflamed area. Neutrophils, however, were capable of moving from reserve compartments into blood in these animals. Furthermore, the functional integrity of their surface membranes, assessed by the capacity of the cells to adhere to nylon fiber, was not altered by alloxan diabetes. An intrinsic cellular defect also did not occur, because the cells were capable of responding to chemotactic stimuli in the Boyden chamber system, provided they were suspended in Eagle's medium or normal serum. Suspended in the corresponding diabetic serum, a blockade of the chemotactic response was observed. Increasing concentrations of diabetic serum, added to a suspension containing neutrophils collected from normal donors, progressively inhibited the response of the cells to a chemotactic stimulus. Hyperglycemia alone or hyperosmolality secondary to hyperglycemia, the presence of ketone bodies, or a direct effect of alloxan did not explain the results. In addition, the capacity to generate chemotactic factors remained intact in diabetic serum. Pretreatment of the diabetic animals with insulin resulted in a gradual recovery of the chemotactic response in vivo and in vitro. We conclude that alloxan-induced diabetic rat serum contains a substance that inhibits neutrophil chemotaxis and that insulin administration is essential for the clearance of this substance from plasma.
Diabetes **36:1307–14, 1987**

Functional changes in the behavior of microvessels (1,2), decreased responsiveness of the endothelial cell to vasoactive agents (3–5), and impaired gross inflammatory reactions (6–10) are described in experimental diabetes mellitus. In many instances the defective responses are acutely reversed by systemic administration of insulin. Conflicting reports, however, are available on the function of polymorphonuclear leukocytes in diabetic subjects. Some studies suggest that the mean chemotactic index in diabetic patients or infants from diabetic mothers is significantly less than in matching controls (11,12). Other investigations report that polymorphonuclear leukocytes from either adult or juvenile diabetic subjects or from patients before and after initiation of insulin treatment are not chemotactically deficient (13,14). In addition, the generation of chemotactic activity from the sera of diabetic subjects is thought to be equivalent to the generation of this activity from the sera of normal subjects (13). Diabetic patients may have a neutrophil phagocytic defect, an impaired intracellular microbicidal effect, or a combined phagocytic and intracellular killing defect (15,16), which are shown as completely (17) or partially (18) reversible by improved diabetic control. Such dysfunctions, if present, may further compromise the inflammatory response and consequently impair the capacity of diabetic patients to resist infection (19–21). This investigation was designed to further examine the effect of diabetes mellitus on polymorphonuclear leukocyte functions in inflammation.

MATERIALS AND METHODS

Diabetes mellitus was induced in 200- to 210-g male Wistar rats with an injection of 40 mg/kg i.v. alloxan dissolved in physiologic saline. Control rats were sham injected with physiologic saline alone. After administration of alloxan, animals were allowed free access to food and water. Thirty days thereafter the presence of diabetes was verified by blood glucose concentrations >200 mg/dl, determined with a blood glucose monitor in samples obtained from the cut tip of the tail. Diabetic animals and matching controls kept

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Received for publication 13 June 1986 and accepted in revised form 15 April 1987.

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in analogous conditions were used in the subsequent 2–3 days after a 4-h interval of fasting. At death a sample of blood was collected from the abdominal aorta while the rat was under ether anesthesia. In this sample, plasma glucose levels were determined by an enzymatic procedure (22), serum immunoreactive insulin was measured by standardized radioimmunoassay (23), and the presence of ketone bodies was qualitatively assessed with the aid of reagent strips in a fashion similar to the testing of urine.

Leukocyte kinetics during development of acute inflammatory response. To induce the accumulation of an inflammatory exudate into the pleural cavity (24), 50 μg carrageenin was dissolved in phosphate-buffered saline (PBS) and injected in a standard volume of 0.1 ml into animals under light ether anesthesia. Before injection and 4 h later, total and differential leukocyte counts were carried out in blood samples obtained from the cut tip of the tail. Immediately after the second blood collection, the animals were reanesthetized with ether, the chest wall was opened, and the inflammatory exudate was withdrawn after washing both pleural spaces with 2 ml PBS containing 25 U/ml heparin. Total and differential leukocyte counts were taken in the exudate. Total leukocyte counts were made in an automated cell counter. Cells in the exudate were washed three times in heparinized PBS before they were counted. Differential leukocyte counts were carried out in stained blood or exudate films.

Neutrophil-adherence assay. The method of Stetcher and Chinea (25) was used. The animals were anesthetized with ether, and the abdominal wall was opened. Blood was collected from the aorta in the presence of preservative-free heparin and kept at room temperature before being placed on the adherence column. This consisted of a 1-ml disposable tuberculin syringe packed to the 0.25-ml mark with 50 mg scrubbed nylon fiber and fitted with a stopcock and 26-gauge disposable needle. The packed columns were preincubated at 37°C for 10 min. After this period of warming, 1 ml of blood was placed on the columns and incubated for 5 min at 37°C. The stopcock was opened, and the blood was allowed to filter through the scrubbed nylon fiber by gravitational force for an additional 20 min. A control aliquot of unfiltered blood was incubated in tubes for the same length of time. The percentage of neutrophils retained on the adherence columns was determined by total and differential (200 cells/slide) leukocyte counts taken of the filtered and unfiltered blood. Leukocyte counts were carried out as described above. For each animal, triplicates were done and the values averaged.

Leukocyte-chemotaxis assay. A slight modification of the method described by Zigmond and Hirsch (26) was used. The acrylic chamber assembly employed for the study of chemotaxis (27) was a cylindrical device (3.8 × 2 cm) consisting of two compartments (0.5 ml each) separated by a 13-mm-diam Millipore filter of 8-μm average pore size and sealed at the circumference by a screw-in ring. The lower compartment received a solution containing the chemotactic agent (activated serum), and the upper compartment received the cell suspension containing 10^6 cells. Endotoxin-activated serum was prepared by incubating 65 μg *Escherichia coli* lipopolysaccharide with 0.1 ml fresh serum of the corresponding cell donor and 0.9 ml Eagle's medium for 30 min at 37°C. To compare the capacity to generate chemotactic factors in sera from normal and diabetic rats, however, varying volumes (0.1, 0.06, and 0.03 ml) of fresh serum were mixed with the same amount of the lipopolysaccharide, the volume of the mixture was completed to 1 ml with Eagle's medium, and incubation was performed as above. Bone marrow cells were employed in the chemotaxis assays due to the impracticability of collecting enough neutrophils from the circulating blood in the rat. The marrow cavity of the femur, severed at both extremities, was washed with 2 ml of Hanks' solution injected with the aid of a syringe. The resulting perfusate contained bone marrow cells that were 39% segmented neutrophils and 29% metamyelocytes. The cells were washed three times in Eagle's medium, their number was automatically determined, and they were resuspended in the same medium or blood serum. The loaded chambers were incubated for 1 h at 37°C. At the end of incubation the filter was removed for fixation and staining. With the micrometer of the fine adjustment of the microscope, the distance was measured from the top of filter to the farthest plane of focus still containing two cells (neutrophils) with a ×40 objective. Twenty fields were counted and averaged for each filter.

Data analysis. Data are expressed as means ± SE of separate experiments. Where appropriate, the data were statistically analyzed by parametric methods (Student's *t* test or analysis of variance).

Materials. Alloxan monohydrate, preservative-free heparin sodium salt, *E. coli* lipopolysaccharide, and bovine crystalline insulin were obtained from Sigma (St. Louis, MO). Carrageenin sodium salt, a polysaccharide composed of sulfated galactose units and of 60,000–100,000 M_r, was from Marine Colloids (Springfield, NJ). NPH insulin was obtained from Lilly (São Paulo, Brazil). Acetylated trypsin was from Mann Research Laboratories (New York), and soybean trypsin inhibitor was from Sigma. Nylon fiber used in adherence assays was from Fenwall Laboratories (Deerfield, IL). Filters used in chemotaxis assays were obtained from Millipore (São Paulo, Brazil). The composition of Hanks' balanced salt solution and Eagle's minimum essential medium was as previously described (28).

TABLE 1
Characteristics of normal adult rats and adult rats given 40 mg/kg alloxan 30 days before

Animals	*n*	Body weight change (g)	Plasma glucose (mg/dl)	Serum insulin (μU/ml)
Normal	72	+105 ± 3	130 ± 4	38 ± 2
Alloxan injected	71	−10 ± 4*	465 ± 14*	9 ± 1*

Values are means ± SE.
**P* < .01 compared with values in respective controls.

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TABLE 2
Blood leukocyte counts in normal and diabetic rats before and 4 h after initiation of acute inflammatory response (pleurisy)

Animals	Cells/mm³				
	Total	Lymphocytes	Monocytes	Neutrophils	Eosinophils
Normal					
Before	16,129 ± 656	10,771 ± 627	680 ± 94	4530 ± 363	148 ± 32
After	24,660 ± 1568*	9738 ± 763	692 ± 95	14,120 ± 1232*	110 ± 15
Diabetic					
Before	14,514 ± 714	8201 ± 475	847 ± 90	5341 ± 368	125 ± 24
After	23,431 ± 1902*	9142 ± 781	913 ± 124	13,275 ± 1330*	101 ± 27

Values are means ± SE. n = 21 for each group.
*P < .01 compared with corresponding values before induction of pleurisy.

RESULTS

General characteristics. The effect of alloxan administration to adult Wistar rats (200–210 g body wt) is shown in Table 1. Estimates were made 30 days after injection and a 4-h interval of fasting. Diabetes caused a significant reduction in body weight gain during this interval. Plasma glucose concentrations were sharply elevated and serum insulin levels were significantly reduced relative to controls. Ketone bodies were present in 80% of alloxan-induced diabetic rats. Approximately 20% of the animals died in the first 10 days after alloxan administration.

Leukocyte kinetics in 4-h carrageenin pleurisy. Blood leukocyte counts, performed immediately before and 4 h after initiation of pleurisy in normal and diabetic rats, showed that similar changes occurred in both groups. An increase of ~53 and 61% in total leukocyte counts was observed in normal and diabetic animals, respectively; this was due to an increase in the number of neutrophils in the circulation. The number of circulating lymphocytes, monocytes, and eosinophils did not change significantly in either group during this interval. The data presented in Table 2 indicate that neutrophils moved into the circulating compartment during the course of an acute inflammatory response to the same degree in normal and diabetic rats. Diabetes, however, markedly reduced the total number of cells migrating into the pleural cavity compared with that of controls. Because the absolute number of mononuclear leukocytes in the exudate was identical in both groups of animals and was virtually indistinguishable from the number of mononuclear cells normally found in the pleural cavity of rats (29), and because the presence of eosinophils in the exudate was extremely rare, a decreased migration of neutrophils accounted for the reduction in the number of cells entering the pleural cavity of diabetic rats during the 4-h carrageenin pleurisy (Fig. 1). Similar results were observed in ketotic and nonketotic animals.

Neutrophil-adherence assay. To assess functional properties of surface membranes, neutrophil adherence to nylon fiber was tested with blood collected 4 h after the injection of carrageenin into the pleural cavity to induce a local inflammatory response and with blood collected from animals that did not bear inflammatory lesions. In both circumstances, analogous results were obtained. No significant difference in the percentage of neutrophils retained on the adherence columns was found between cells from normal and diabetic donors (Fig. 2).

Leukocyte-chemotaxis assay. To evaluate the capacity of normal and diabetic sera to generate chemotactic factors, cells from normal animals were suspended in Eagle's medium and placed in the upper compartment of the test chamber. The bottom compartment of the chamber was filled with a mixture of varying concentrations of normal or diabetic sera, diluted in Eagle's medium and previously incubated with a fixed amount of *E. coli* lipopolysaccharide (activated serum). Migration of cells into the filter was proportional to the concentration of sera present in the mixture. However, for each concentration of both sera, equivalent cell responses were found (Fig. 3). A 10% dilution of activated serum was used in the following experiments.

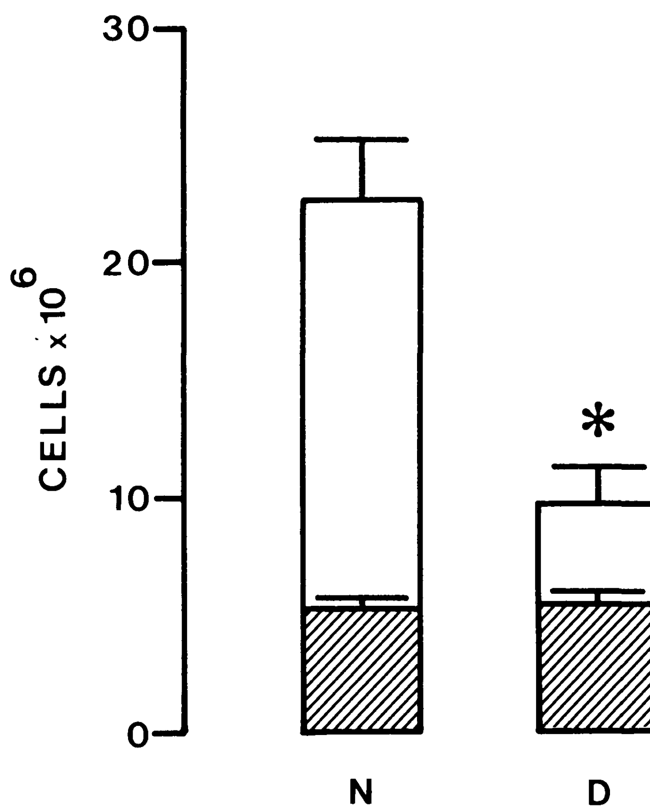

FIG. 1. Polymorphonuclear (*open area*) and mononuclear (*hatched area*) leukocytes in inflammatory exudate (pleurisy) of normal (N) and diabetic (D) rats. Values are means ± SE. n = 21 in each group. *P < .01 compared with values in respective controls.

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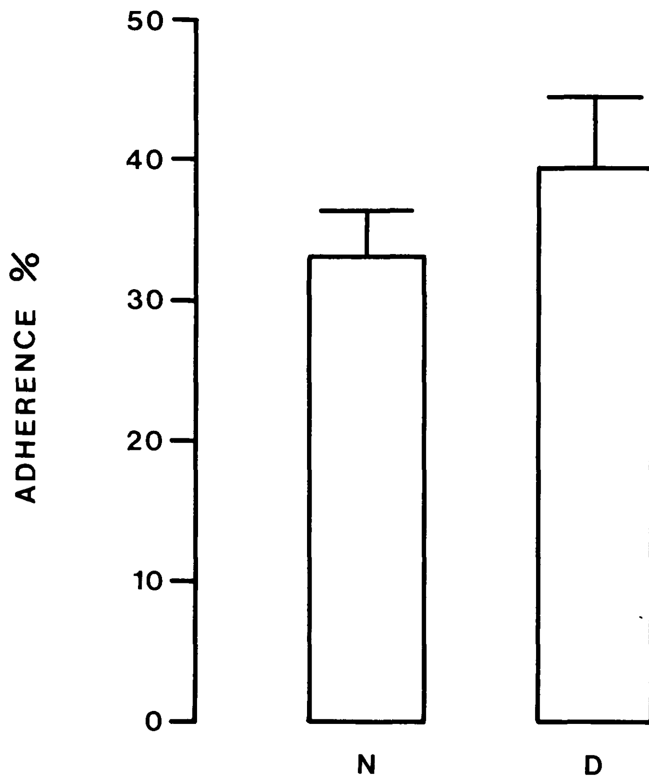

FIG. 2. Neutrophil adherence to nylon fiber in normal (N, n = 10) and diabetic (D, n = 9) rats. Values are means ± SE. Differences were not statistically significant.

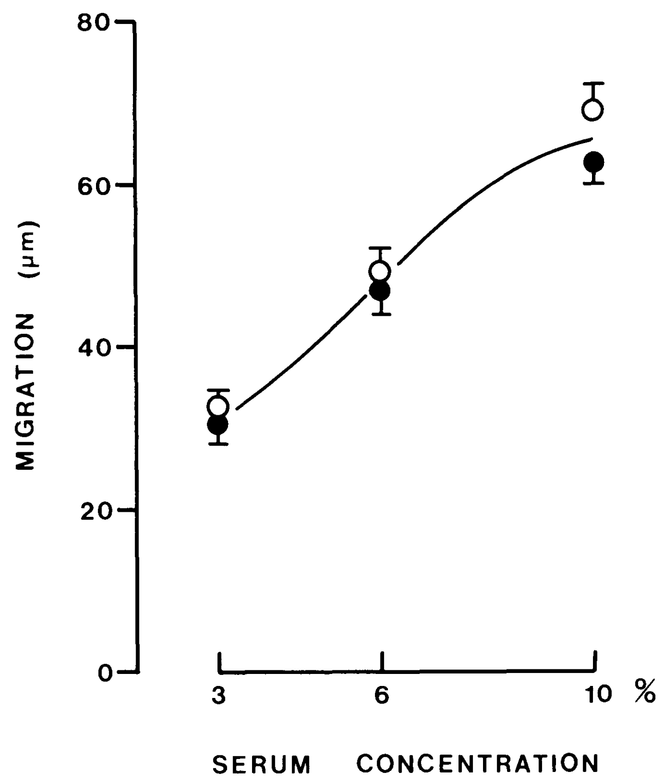

FIG. 3. Chemotactic tests (migration into filter) for cells from normal rats in Boyden chamber system. Chemotactic factors were generated from varying concentrations of normal (○) or diabetic (●) sera. Values are means ± SE of 8 experiments in each group.

To compare the chemotactic response of cells from normal and diabetic animals, generation of chemotactic factors for each animal was induced in its own serum. Endotoxin-activated serum stimulated cells from both groups of animals to migrate into the filter, provided the cells were suspended in Eagle's medium or normal serum. A partial blockade of movement into the filter resulted when cells were suspended in diabetic serum, regardless of whether donors were normal or diabetic animals (Table 3). The blockade of migration was not the result of the increased concentration of glucose, because addition of glucose to the medium in which cells from normal animals were suspended did not affect penetration into the filter, and because cells obtained from diabetic animals and suspended in blood serum of the corresponding donor moved less into the filter even when addition of glucose to the bottom compartment of the Boyden chamber resulted in equal concentrations above and below the filter (Table 4). Accordingly, increased concentrations of glucose in the suspending medium or addition of glucose to the bottom compartment of the Boyden chamber to avoid possible glucose concentration gradients due to the presence of diabetic serum in the upper compartment did not appear to influence cell migration.

Effect of increasing concentrations of diabetic serum on normal leukocyte chemotaxis. Increasing concentrations of diabetic serum added to the cell suspension progressively inhibited chemotaxis of leukocytes from normal donors (Fig. 4). A mixture of 30% diabetic and 70% normal serum depressed chemotaxis by ~21%. A mixture of equal parts of diabetic and normal sera produced a reduction of ~42% in

TABLE 3
Chemotactic tests for cells from normal and diabetic rats in the Boyden chamber system

Cell donors	n	Top compartment	Bottom compartment	Migration into filter (μm)
Normal	14	Eagle's medium	Eagle's medium	20.0 ± 1.1
	11	Eagle's medium	Activated serum*	72.1 ± 3.8
	17	Corresponding serum	Activated serum	61.3 ± 1.8
	7	Diabetic serum	Activated serum	46.3 ± 2.7†
Diabetic	11	Eagle's medium	Eagle's medium	20.4 ± 0.9
	8	Eagle's medium	Activated serum	79.2 ± 1.3
	17	Corresponding serum	Activated serum	37.6 ± 2.0
	8	Normal serum	Activated serum	66.0 ± 4.0†

Cells were placed in top compartment of the Boyden chamber. Values are means ± SE.
*Always from corresponding cell donor.
†$P < .01$ compared with values obtained when cells were suspended in corresponding serum.

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TABLE 4
Chemotactic tests for cells from normal and diabetic rats in the Boyden chamber system: effect of glucose addition

Cell donors	*n*	Top compartment	Bottom compartment	Migration of filter (μm)
Normal	11	Eagle's medium	Activated serum*	72.1 ± 3.8
	7	Eagle's medium + glucose†	Activated serum	66.4 ± 3.0
Diabetic	7	Corresponding serum	Activated serum	38.7 ± 1.5
	7	Corresponding serum	Activated serum + glucose‡	42.7 ± 1.2

Cells were placed in top compartment of the Boyden chamber. Values are means ± SE. No significant difference was found between values within each group of cell donors.
*Always from corresponding cell donor.
†Final concentration 500 mg/dl.
‡Added to match corresponding blood glucose concentrations.

chemotaxis. Maximal inhibition (50%) was obtained with a mixture containing 70% diabetic and 30% normal serum. The chemotactic response of cells suspended in 100% normal serum was equivalent to that of cells suspended in Eagle's medium alone.

Failure of insulin in vitro to reverse diabetic serum effects. Leukocytes from diabetic animals were suspended in the corresponding serum and crystalline insulin was added to the cell suspension in concentrations up to 1 mU/ml. Results indicated that insulin in vitro was not capable of reversing the depressor effect of diabetic serum on leukocyte chemotaxis. Values obtained for cell migration into the filter in the absence and presence of 1 mU/ml crystalline insulin were 36.6 ± 1.4 and 35.4 ± 1.2 μm, respectively.

Reversal of the chemotaxis defect by pretreatment of diabetic animals with insulin. Diabetic rats were given 2–3 U s.c. NPH insulin each evening for 3, 10, or 22 days. Insulin treatment started 27, 20, or 8 days after alloxan treatment, respectively, and cells were collected 18 h after the last dose of insulin. Suspended in the corresponding serum, which previously blocked their migration into filter, leukocytes obtained from treated animals exhibited a gradual recovery of the chemotactic response. In parallel, migration of leukocytes in vivo to an inflamed area (pleurisy) gradually

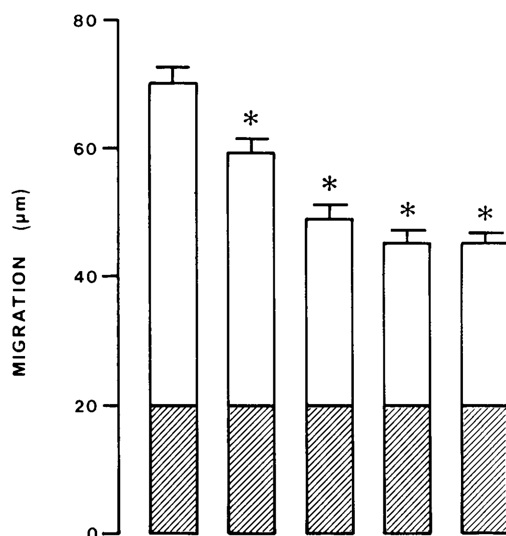

FIG. 4. Chemotactic tests (migration into filter) for cells from normal rats in Boyden chamber system. Results were obtained when cells were suspended in increasing concentrations of diabetic serum. Each *bar* is mean ± SE of 7 experiments. *Hatched areas* indicate average migration in absence of chemotactic factors. $P < .05$ compared with values obtained in absence of diabetic serum.

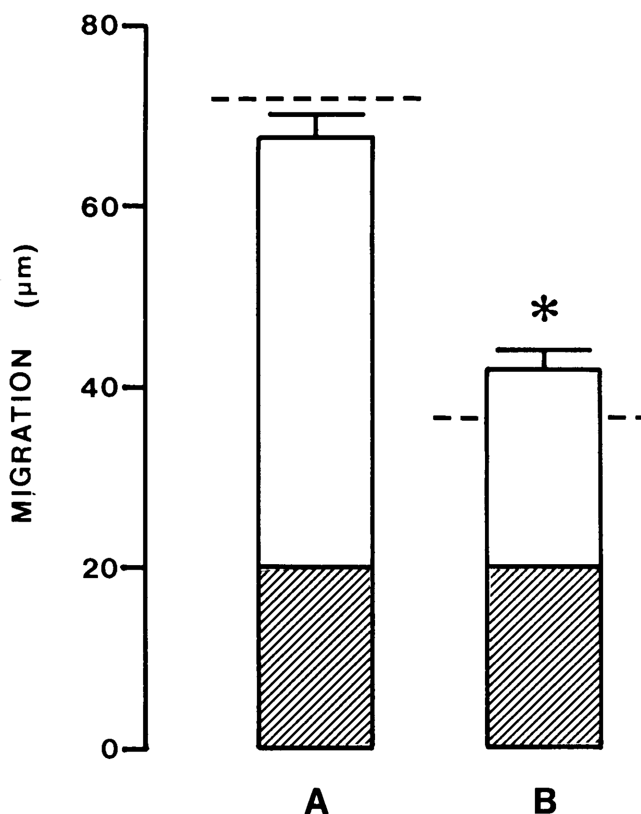

FIG. 5. Chemotactic tests for cells from diabetic rats in Boyden chamber system. Results were obtained when cells collected from animals on insulin treatment for 3 days were mixed with serum from diabetic animals on insulin treatment for 22 days (*A*) or when cells from animals on insulin treatment for 22 days were mixed with serum from diabetic animals on insulin treatment for 3 days (*B*). *Dashed lines* indicate chemotactic values for cells from diabetic rats kept on insulin treatment for 22 or 3 days, respectively, and tested in presence of original serum. *Hatched areas* denote average migration in absence of chemotactic factors. NPH insulin (2–3 U/day) was used. Each *bar* is mean ± SE of 6 experiments. *$P < .01$ compared with values in *A*.

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TABLE 5
Effect of insulin treatment on general characteristics of diabetic rats and on chemotactic response of their blood cells

Animals	Body weight change (g)	Plasma glucose (mg/dl)	Chemotactic response* (migration into filter) (μm)	Cells × 10^6 in inflammatory exudate†		
				Polymorphic	Mononuclear	*n*
Normal	+108 ± 5 (20)	130 ± 9 (20)	62.8 ± 1.8 (10)	25.60 ± 2.20	6.10 ± 0.64	10
Diabetic + 3 days insulin‡	−15 ± 6§ (12)	352 ± 31§ (12)	37.0 ± 2.1§ (6)	12.29 ± 3.00§	5.55 ± 1.22	6
Diabetic + 10 days insulin‡	+41 ± 6§ (14)	315 ± 28§ (14)	45.4 ± 2.5§ (6)	17.52 ± 3.10§	5.91 ± 1.32	8
Diabetic + 22 days insulin‡	+53 ± 5§ (17)	325 ± 27§ (17)	71.6 ± 2.2 (10)	28.77 ± 3.43	5.83 ± 1.23	7

Values are means ± SE. Number of animals is shown in parentheses.
*Cells, placed in top compartment of the Boyden chamber, were suspended in corresponding serum. Bottom compartment was loaded with activated serum of same animal in Eagle's medium, as in previous experiments.
†Obtained from pleural cavity 4 h after injection of 50 μg carrageenin.
‡NPH insulin, 2–3 U s.c., was given each evening for 3, 10, or 22 days; treatment started 27, 20, or 8 days after alloxan treatment. Normal animals received same volume of saline by same route.
§$P < .01$ compared with value in respective control.

improved in insulin-treated animals, up to a complete reversal of the inhibited response in the group receiving insulin for 22 days. Because a single daily dose of NPH insulin was used and glucose levels were determined ~18 h later, it is not surprising that mean glucose levels were still high in treated animals. However, a gradual amelioration of the diabetic state occurred with treatment, indicated by body weight gain and the absence of ketone bodies in the serum of these animals. Results are presented in Table 5.

To further investigate the effect of diabetic serum on leukocyte chemotaxis, cells from animals started on insulin on day 27 after the administration of alloxan were suspended in serum from animals started on insulin treatment on day 8 after alloxan treatment. Reversal of the previously inhibited chemotactic response was observed in this condition. Conversely, leukocytes from animals started on insulin on day 8 after the administration of alloxan mixed with serum from animals started on insulin treatment on day 27 after alloxan treatment exhibited reduced chemotactic responses. The finding strongly suggests that the defect in chemotaxis is due to a serum inhibitor (Fig. 5).

TABLE 6
Chemotactic tests for cells from normal rats in the Boyden chamber system: effect of heating, dialysis, and incubation with trypsin on inhibitory activity of diabetic serum

Serum samples*	Migration into filter (μm)	
	Normal rats	Diabetic rats
Unheated	72.0 ± 2.9	37.1 ± 2.0
Heated†	86.0 ± 3.2	74.3 ± 3.0‖
Nondialyzed	70.2 ± 1.9	36.1 ± 2.4
Dialyzed‡	69.4 ± 2.5	36.3 ± 2.2
Undigested	65.2 ± 1.8	35.5 ± 2.1
Digested§	80.8 ± 4.0	75.8 ± 2.9‖

Values are means ± SE. $n = 5$ in each group.
*Serum from each animal was divided into 2 samples. One was subjected to either procedure indicated; the other was control. Cells were suspended in samples and placed in top compartment of chambers. Bottom compartment was loaded with activated normal serum.
†Heating for 1 h at 56°C.
‡Against Hanks' solution during 16 h at 4°C in proportion of 1:1000 (vol/vol), with 10,000-M_r retention dialysis tubing.
§Incubation with 200 μg/ml trypsin for 30 min at 37°C, followed by addition of 300 μg/ml soybean trypsin inhibitor and reincubation for 30 min under same conditions.
‖$P < .01$ compared with matching controls.

Partial characterization of the inhibitory factor in diabetic serum. The effect of heating, dialysis, and incubation with trypsin on the inhibitory activity of diabetic serum was investigated. Samples were heated at 56°C for 1 h and tested. Dialysis was performed with 10,000-M_r retention dialysis tubing. Enzymatic stability of the samples was assayed after incubation with trypsin and subsequent addition of a trypsin inhibitor. Cells from normal donors were used for the chemotactic tests. Complete loss of the inhibitory activity was observed on heating and after incubation with trypsin but not after dialysis of the samples, as shown in Table 6.

DISCUSSION

Alloxan-induced diabetic rat serum appears to contain a substance that can produce inhibition of neutrophil chemotaxis based on the following observations. First, the early local exudative cellular reaction in an inflammatory lesion was impaired in alloxan-induced diabetic animals due to a reduced migration of neutrophils to the inflamed area. Neutrophils, however, were capable of moving from reserve compartments into blood as much in normal as in diabetic animals. The characteristic neutrophilia observed during the early stages of an inflammatory response was therefore comparable in normal and diabetic rats. Second, increasing concentrations of diabetic serum added to a suspension containing neutrophils collected from normal donors progressively inhibited the response of the cells to a chemotactic stimulus. Maximal inhibition, obtained when cells were suspended in diabetic serum alone, was of ~50% relative to controls. Third, an intrinsic cellular defect did not occur, because neutrophils obtained from diabetic animals were still capable of responding to chemotactic stimuli in a normal fashion, provided they were suspended in Eagle's medium or normal serum. Furthermore, the functional integrity of neutrophil surface membranes, assessed by the capacity of the cells to adhere to nylon fiber, was not altered by alloxan-induced diabetes.

That hyperglycemia and hyperosmolality secondary to hyperglycemia were not relevant factors was indicated by the finding that addition of glucose to the suspending media did

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not affect chemotaxis of neutrophils collected from normal donors. Similarly, the eventual occurrence of glucose concentration gradients between intra- and extravascular fluids seemed irrelevant, because addition of glucose to the bottom compartment of the test chamber to obtain equivalent concentrations above and below the filter did not improve the chemotactic response of the cells suspended in diabetic serum. The depressor effect of diabetic serum on neutrophil chemotaxis was also unrelated to the presence of ketone bodies. This was shown by an equivalent reduction in the number of cells in the inflammatory exudate of ketotic and nonketotic animals. In addition, the same capacity to generate chemotactic factors after incubation with *E. coli* polysaccharide was observed in sera from normal and diabetic rats. Varying concentrations of normal and diabetic sera yielded equivalent chemotactic activity on incubation with the lipopolysaccharide. A direct effect of alloxan is improbable due to its extremely short half-life (<1 min in blood; 30).

These results indicated that a depressed cellular response to nonspecific inflammatory stimuli is produced during an insulin-deficient diabetic state and suggested that this is due to the presence of an inhibitory factor in plasma. The early granulocyte phase of the local cellular response in surgically abraded lesions is significantly delayed and diminished in poorly controlled diabetic patients (31). Furthermore, reduced cellular immune responses are also reported as a consequence of the production of circulating inhibitory factor(s) in diabetic rat plasma. This reduction is not the result of a direct cytotoxic effect of alloxan-induced diabetic plasma on mononuclear leukocytes and cannot be explained by hyperglycemia alone (32). In addition, phagocytic defects and reduced intracellular microbicidal effects are common findings in diabetic subjects (11,15,18,31,33–37), despite some conflicting results (13,14,38). Coupled with characteristic impaired responses of microvessels to inflammatory stimuli (1–8), defective leukocyte functions in diabetes mellitus thereby represent an aggravating factor for host defense in the first stages of infection.

The susceptibility of diabetic subjects to infection generally applies to uncontrolled diabetes, because with adequate treatment the response of the diabetic subject to infection approaches normal. The inability of insulin in vitro to reverse diabetic serum effects on neutrophil migration and the gradual recovery of the animals maintained on insulin treatment further evidenced the presence of a circulating inhibitory substance of chemotaxis in diabetes mellitus. Treatment of the animals with insulin, however, did not lead to a strictly controlled metabolic state of the animals, as indicated by the remaining hyperglycemia that, nevertheless, was of a lower magnitude than in untreated animals. Diabetes mellitus therefore decreases the capacity of the animals to respond to inflammatory stimuli not only because of functional changes in the behavior of microvessels but also because the early local exudative cellular reaction in an inflamed area is impaired. This appears to be due to the presence of an inhibitory factor in the serum of these animals. The inhibitory factor was shown to be heat labile (56°C), destroyed by incubation with trypsin, and retained after dialysis with 10,000-M_r retention dialysis tubing. This information provides good evidence that the inhibitory activity is associated with protein. Counterregulatory peptide hormones, which are increased in diabetic serum, might contribute to such activity, because they are not expected to be neutralized by the addition of pharmacologic amounts of insulin in vitro. Finally, the experiments seem to indicate that treatment with insulin is essential to correct the vascular and cellular responses to injury and therefore to improve host defense against infection.

ACKNOWLEDGMENTS

The expert secretarial assistance of Yara Corradini is gratefully acknowledged. We are indebted to the Laboratory of Carbohydrates and Radioimmunoassay, School of Medicine, University of São Paulo, for help in serum insulin determinations and to Dr. Elizabeth S. Oliveira, in our department, for help with dialysis experiments.

This work was supported in part by Financiadora de Estudos e Pesquisas (FINEP), Brazil.

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