Diabetes and Neutrophil Chemotaxis

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SUMMARY

The chemotaxis of PMN cells from adult and juvenile diabetics and proper control subjects was found to be comparable. Similarly, chemotactic activity generated from diabetic sera was not different from the activity generated from the normal sera. DIABETES *26*:466-68, May, 1977.

It has repeatedly been stated that patients with diabetes mellitus are more susceptible to bacterial and fungal infections than normal people. This has led to a variety of investigations designed to clarify the mechanisms that would account for this. Abnormalities of leukocyte mobilization in Rebuck skin windows, independently from or associated with ketoacidosis,[1,2] abnormal in-vitro polymorphonuclear (PMN) mobilization and phagocytosis in diabetic children prior to insulin treatment,[3] and reduced PMN phagocytic activity in diabetics with ketoacidosis[4] have been described. Studies in diabetic rats and rabbits have indeed shown some increase in susceptibility to bacterial and fungal infections,[5,6] and in rats with alloxan-induced diabetes impaired PMN phagocytosis has been documented.[6]

Recently another defect in PMN function, abnormal chemotaxis, has been observed in leukocytes of diabetic patients.[7-9] Furthermore, plasma or sera of children with diabetes were found to be deficient in their ability to produce chemotactic activity.[8] However, these findings have recently been challenged.[10]

The present study was undertaken to assess the PMN chemotactic function in adult and juvenile diabetics as well as to measure the chemotactic activity generated by the serum of these patients. The results indicate that PMNs from neither adult nor juvenile diabetics are chemotactically deficient, and the generation of chemotactic activity from the sera of the diabetics is not different from the generation of chemotaxis from the sera of normal subjects.

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MATERIAL AND METHODS

Blood was obtained from juvenile and adult diabetics and appropriate control subjects into heparinized tubes. White blood cells were separated with a mixture of Methocel-Isopaque[11] and washed with tissue culture medium 199 (TC-199). A final suspension of 2.5×10^6 PMN per milliliter in 2 per cent bovine albumin was made.

Chemotactic factor was generated from sera of diabetics as well as normal subjects by incubating 0.1 ml. of sera with 1 mg. of Zymosan and then bringing up the total volume to 1 ml. with TC-199.[12,13]

The chemotaxis was measured by a previously described modification of the Boyden assay.[13,14] In brief, 2.5×10^6 PMN in 2 per cent bovine albumin was placed in the upper compartment of the modified Boyden chambers and separated from the chemotactic agent by means of Micropore filters (pore size of 5 μ). After three hours of incubation at 37° C. the chambers were disassembled and the filters separated, fixed, and stained by routine histologic methods.[13] They were then examined under the microscope and final assay was made by taking the average measurements in five random fields of (a) the distance traveled from the top of the filter to the furthest plane by at least two cells and (b) the number of neutrophils found on the lower surface of the filter. Similar measurements were made in filters obtained from control chambers without chemotactic factors. The difference between the two values was reported as final chemotaxis.

RESULTS

A total of 21 juvenile (11 males and 10 females) and 26 adult diabetics (12 males and 14 females) together

with 18 juvenile (eight males and 10 females) and 18 controls (nine males and nine females) were studied. The mean age of juvenile diabetics (11.6, S.D. 3.6 years) and juvenile controls (9.9, S.D. 3.9 years) as well as the mean age of adult diabetics (48.5, S.D. 11.9 years) and adult controls (49.8, S.D. 13.2 years) were comparable.

The chemotactic activity of PMN leukocytes from adult diabetics and normal adult controls as well as that of juvenile diabetics and juvenile controls were comparable. When the distance traveled by the PMN through the Micropore filter were measured, again no appreciable differences were elicited between the adult and juvenile diabetics versus their appropriate controls. These results are summarized in table 1. The chemotactic activity generated by the sera of adult as well as juvenile diabetics were comparable to the chemotactic activity generated by the sera of adult and juvenile controls.

DISCUSSION

Our studies failed to demonstrate any defect in chemotaxis of the PMN obtained from adult or juvenile diabetics. Furthermore, sera from such patients were not deficient in their ability to generate chemotactic activity.

The duration of diabetes in the juvenile diabetics was from one month to 16 years and in adult patients from three to 30 years. All juvenile diabetics have been on insulin therapy since the inception of the disease. Some of the adult diabetics were on oral medications, and three were controlled on diet alone. Ketoacidosis was present in only one of the juvenile diabetics. No association was found between the chemotactic activity and the duration of the disease, kind of treatment or lack of it, or the one instance of diabetic ketoacidosis.

The discrepancy between our findings and those of others[7,8] may be partially explained by differences in technique. Zymosan, used in our experiments to generate chemotactic factors from the serum, works primarily through the alternate complement pathway, whereas antigen-antibody complexes used by others act through the classic pathway. However, antibodies against Zymosan are known to exist in many normal and diabetic subjects. In these, the action of Zymosan follows the classic as well as the alternate pathway. If diabetics are more susceptible to local or systemic infections, our results indicate that abnormal PMN chemotaxis is not the cause of it, and the basic defect must lie elsewhere. However, the concept that diabetics are more susceptible to infection can no longer be accepted as an unqualified fact. The few objective studies available[15,16] have shown that the actual incidence of infections in well-controlled diabetics is no greater than the incidence in the normal population.

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TABLE 1

Final chemotactic activity of PMN

	Number of PMN/HPF Mean ± S.E. (range)	P†	Distance traveled in μ Mean ± S.E. (range)	P†
Adult diabetics (26)*	94.0 ± 13.8 (11-231)		44.5 ± 2.9 (19-72)	
		>0.2		>0.2
Adult controls (18)	69.5 ± 13.6 (15-195)		39.1 ± 3.2 (18-71)	
Juvenile diabetics (21)	43.0 ± 6.8 (11-113)		50.3 ± 2.1 (31-65)	
		>0.5		>0.5
Juvenile controls (17)	40.0 ± 4.7 (11- 85)		52.4± 3.2 (24-72)	

*Number of subjects tested is in parentheses.
†Paired sample Student's *t* test.

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