Impaired Granulocyte Function in Patients with Diabetes Mellitus

Hideaki KANESHIGE, Masayuki ENDOH, Yasuhiko TOMINO, Yasuo NOMOTO, Hideto SAKAI and Shigeru ARIMORI

Department of Internal Medicine, School of Medicine, Tokai University
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Phagocytosis and intracellular killing of Staphylococcus aureus by granulocytes were examined in diabetic patients. There was no significant difference in the phagocytic activity of granulocytes between control and diabetic subjects. However, intracellular killing by granulocytes was significantly reduced in insulin-treated diabetic patients compared with control subjects. No significant difference was observed between controls and diet-treated diabetic patients.

It is suggested that decreased activity of intracellular killing of bacteria in granulocytes is one of the mechanisms of increased susceptibility to infection in patients with advanced stages of diabetes mellitus.

(Key Words: Diabetes Mellitus, Phagocytosis, Intracellular Killing)

INTRODUCTION

It is generally assumed that patients with diabetes mellitus are more susceptible to infection than normal controls (5). Since recurrent bacterial infections in diabetic patients are presumed to be associated with defective granulocyte functions, several hypothesis have been proposed to explain the mechanism of impaired granulocyte functions in diabetic patients (2, 4, 6, 7).

The killing of bacteria by granulocytes can be classified into several stages: opsonization of the particles by serum factors, attachment of the opsonized particles to the cell surface, engulfment on such particles, intracellular killing of microorganisms and digestion of microorganisms.

This study was designed to examined the activity of phagocytosis and intracellular killing by granulocytes from diabetic patients.

MATERIAL AND METHODS

1) Patient selection

Eight patients with maturity onset diabetes mellitus without recent histories of infections or ketoacidosis were chosen at random from our Diabetes Clinic. Among these diabetic patients four were treated with insulin and four were controlled by diet therapy. Three healthy adults including hospital personnel were used as control subjects.

HIDEAKI KANESHIGE. Department of Internal Medicine, School of Medicine, Tokai University, Bohseidai, Isehara, Kanagawa 259—11, Japan
2) **Separation of granulocytes**

The venous blood was collected in a sterile tube containing phenol-free heparin solution. The erythrocytes were sedimented for 30 min at 37°C. The leucocyte-rich supernatent fluid was layered over Ficoll-Conray solution (specific gravity: 1.077) and centrifuged at 400g for 30 min. The sedimented leucocytes were washed twice and suspended in RPMI medium 1640 (GIBCO, Grand Island, New York).

3) **Opsonins**

Blood from the same donar was collected in non-heparinized tubes and serum was obtained after clotting. Inactivated serum was prepared by heating in a 56°C water bath for 30 min.

4) **Microorganisms**

Clinical isolates of Staphylococcus aureus were obtained from the clinical microbiology laboratory in Tokai University Hospital.

5) **Phagocytosis**

Mixtures consisting of 1ml of $1.0 \times 10^7$ washed granulocytes, 1ml of 1.0ml of bacteria and 0.2ml serum were prepared and incubated for two hours in 5% CO$_2$ air at 37°C under continuous rotation. The mixtures were centrifuged and the supernatants were diluted serially in saline and aliquots of 0.1ml of the three highest dilutions were pipetted onto blood-agar plates and immediately spread with a fine wire loop. The plates were incubated at 37°C in CO$_2$ air for 24 hours and colonies were counted.

6) **Intracellular killing**

After the phagocytosis of bacteria, the bacteria-cell suspension was centrifuged and the supernatant fluid was removed. The sedimented granulocytes were washed twice with RPMI 1640 and lysed by osmotic disruption. Serial ten-fold dilutions in saline were made from the samples and the number of viable bacteria was determined by colony count.

**RESULTS**

The phagocytosis of Staphylococcus aureus by granulocytes from the eight diabetic patients was not significantly different between diabetic patients and controls ($p > 0.25$) (Fig. 1). However, the intracellular killing of S. aureus by granulocytes was significantly reduced ($p < 0.05$) in the insulin-dependent diabetic patients. Conversely, no significant difference was observed between controls and diet-treated patients ($p > 0.25$) (Fig. 2).

**DISCUSSION**

Various investigaters have employed many different assay techniques for investigating phagocytic activity in diabetic patients. Bybee and Rogers (4) observed impaired phagocytosis of staphylococcus by neutrophils obtained from 17 diabetic patients with ketoacidosis and this impairment improved after correction of acidosis. Furthermore, a defect in phagocytosis in the non-ketotic state in diabetic patients was demonstrated in poorly controlled diabetic patients (1, 2). The disturbances in phagocytosis and intracellular killing were improved in patients with poorly controlled...
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Fig. 1 Phagocytosis of Staphylococcus aureus by control and diabetic granulocytes

Fig. 2 Intracellular killing of Staphylococcus aureus by control and diabetic granulocytes.
diabetes after treatment with insulin (3, 6). Miller and Baker (5) reported that juvenile diabetic polymorphonuclear leucocytes showed normal phagocytosis with decreased chemotactic activity.

Results from our present study show that granulocytes from the diabetic group showed normal phagocytosis, whereas impaired intracellular killing was observed in insulin-treated patients. Although the mean phagocytic activity fell in diabetic groups when compared with normal controls, the reduced level was not statistically significant. This observation confirmed that phagocytosis is not impaired in diabetic patients without ketoacidosis. Bagdade and Root (2) reported that diabetic subjects demonstrated a marked impairment in phagocytosis before therapy which improved remarkably after antidiabetic therapy. Despite this improvement, the microbicidal rate remained below control levels. In their study on non-ketotic diabetic patients, abnormal phagocytosis was apparent only in patients with overt signs of insulin deficiency. These findings imply that host defense mechanisms have a reversibility in diabetic patients after control of their diabetes.

The normal phagocytic activity associated with impaired intracellular killing in granulocytes in insulin-treated patients might be due to partial impairment of the diabetic condition in these patients since they had been fairly well controlled by insulin.

It is concluded that good control of diabetes appears to be important for minimizing the susceptibility of infections in patients with diabetes mellitus.

REFERENCES