Impairment of Polymorphonuclear Leukocyte Function and Metabolic Control of Diabetes

WILHELM MARHOFFER, MD
MICHAEL STEIN
ERIKA MAESER, MD
KONRAD FEDERLIN, MD

OBJECTIVE — In this study, ingestion of *Staphylococcus aureus* and "bacteria killing" (BK) were measured to evaluate polymorphonuclear leukocyte (PMN) phagocytic functions and chemiluminescence response (CL) to phorbolmyristic acetate (PMA) as respiratory burst activity with regard to metabolic control parameters in diabetic patients.

RESEARCH DESIGN AND METHODS — PMN phagocytic functions were assessed in 40 diabetic patients, all receiving insulin and in poor metabolic control, with ^{3}H-thymidine–labeled *Staphylococcus aureus* in a modified radiometric assay. Bacteria killing was determined by pure-plate counting of surviving bacteria (colony-forming units [cfu]) and luminol-enhanced CL in response to PMA as a measure of respiratory burst. PMN function data were correlated to HbA_1 as parameter of recent metabolic control.

RESULTS — PMN of diabetic patients showed a significant reduction in *Staphylococcus aureus* (50.7 ± 4.1%) and BK (29.4 ± 4.2%) compared with healthy nondiabetic control subjects (76.6 ± 4.6% and 16.3 ± 3.1%, respectively, $P < 0.001$), and PMN CL response was markedly reduced in diabetic patients also. Linear regression analysis showed a highly significant negative correlation of HbA_1 versus *Staphylococcus aureus* ($r = -0.67$, $P = 0.001$) and a positive correlation for BK ($r = 0.73$, $P < 0.001$). This was also true for CL, although this did not reach statistical significance ($P = 0.06$).

CONCLUSIONS — The data obtained demonstrate impaired PMN phagocytic functions and CL response in diabetic patients. These findings suggest inhibitory effects of elevated glucose concentrations on PMNs, a possible role of protein glycosylation for impairing PMN function, thus contributing in part to altered host defense.

FROM THE THIRD MEDICAL CLINIC AND POLICLINIC, UNIVERSITY OF GIESSEN, GIESSEN, GERMANY.

ADDRESS CORRESPONDENCE TO DR. WILHELM MARHOFFER, THIRD MEDICAL CLINIC AND POLICLINIC, UNIVERSITY OF GIESSEN, RODTHOHL 6, 6300 GIESSEN, GERMANY.

RECEIVED FOR PUBLICATION 7 JANUARY 1991 AND ACCEPTED IN REVISED FORM 3 JULY 1991.

Bacterial infections in diabetic patients are an important cause of increased morbidity and mortality in diabetes (1–4). Polymorphonuclear leukocytes (PMNs) play an important role in host defense, and impaired PMN function has been demonstrated in poorly controlled diabetic subjects with ketoacidosis (5,6). Normal respiratory burst activity of PMNs for microbial defense is either normal or variably reduced in diabetic patients (7,8), but the mechanisms of altered cell function is not well understood. In previous studies, PMN dysfunction was described but the metabolic control of diabetes was not well characterized (9–18). In this study, we evaluated PMN ingestion and bacterial killing for phagocytic activity and luminol-enhanced chemiluminescence (CL) in response to phorbolester stimulation for oxidative burst activity regarding levels of blood glucose and HbA_1 as a measure of diabetes control.

RESEARCH DESIGN AND METHODS

— The study population consisted of 40 diabetic patients (20 insulin dependent, 20 non–insulin dependent, all receiving insulin. The patients were followed as outpatients in our clinic. The control group was comprised of 30 healthy nondiabetic adults corresponding in age and sex to the patient group. Clinical data of patients are summarized in Table 1. None of the patients or control subjects had clinical evidence of infection at the time of study. The study protocol was approved by the University of Giessen ethic committee, and all participants gave informed consent.

Dextran 70 (Fresenius, Homburg, Germany) was diluted in 0.9% saline to 6% solution. Phorbol myristic acetate (PMA; 1.62×10^{-9} M), for initiating PMN respiratory burst, was dissolved in dimethyl sulfoxide (DMSO); 6×10^{-6} M luminol (22)

Table 1—*Clinical data of diabetic and control groups*

	Insulin dependent	Non–insulin dependent	Control
N (F/M)	12/8	11/9	17/13
Duration of diabetes (yr)	10.6 (0.5–35)	8.3 (0.5–26)	
HbA_1 (%)	12.1 (8.8–17.1)	11.4 (8.9–15.6)	6.8 (5.6–7.8)
Age (yr)	28 (16–51)	62 (54–75)	37 (21–64)

Ranges in parentheses.

was prepared in phosphate-buffered saline (PBS) containing 40×10^{-6} M triethylamine. DMSO and triethylamine were diluted at least 1:100, and neither altered PMN activation at this concentration.

PMNs were isolated from venous blood anticoagulated with 1 U/ml heparin via density centrifugation after erythrocyte gravity sedimentation in 6% dextran at room temperature (19). After lysis of contaminating erythrocytes by hypotonic shock, the leukocytes were washed and adjusted to 1×10^7/ml. Viability was assessed by trypan blue exclusion, which yielded at least 93% viable PMNs.

Staphylococcus aureus K 807 (kindly provided by Prof. H.-G. Blobel, Institute of Veterinary. Pathology, Univ. of Giessen), stored on blood agar at 4°C, was grown overnight at 37°C in trypsin soy broth supplemented with 0.05 μCi ^{3}H-thymidine (sp act 5 mCi/mmol, Amersham, UK). Before use, bacteria were opsonized with 10% pooled normal human serum from 10 healthy nondiabetic donors for 30 min at 37°C, washed twice with PBS (pH 7.4), suspended to 1×10^9/ml, and kept on ice until use.

PMN ingestion of ^{3}H-thymidine–labeled *Staphylococcus aureus* was measured according to Peterson et al. (20), with slight modifications. Phagocytosis was calculated after removing non–phagocyte-associated bacteria via differential centrifugation as percentage of PMN uptake of total added radioactivity percentage uptake = (cpm A/cpm B) × 100.

To determine bacterial killing, the phagocytosis mixture was stopped with PBS, and 20-μl samples were transferred to 10 ml distilled water to lyse PMNs by osmotic shock. Aliquots (100 μl) were inoculated on cysteine lactose electrolyte deficient agar and surviving bacteria (colony-forming units [cfu]) were determined (pure-plate counting). Results are percentage of surving bacteria (%cfu).

For CL measurements, Biolumat (model LB 9500 C, Berthold, Wildbad, Germany) was used. The samples to be analyzed at 37°C contained 20 μl PMN, 20 μl luminol, and 160 μl PBS for background CL recording (37°C). PMN stimulation was performed by adding PMA (final conc 8.1×10^{-12} M). Experiments were performed over 60 min, and results are expressed as relative light units/minute.

All assays were run in triplicate. Statistical analysis was done by two-way analysis of variance with repeated-

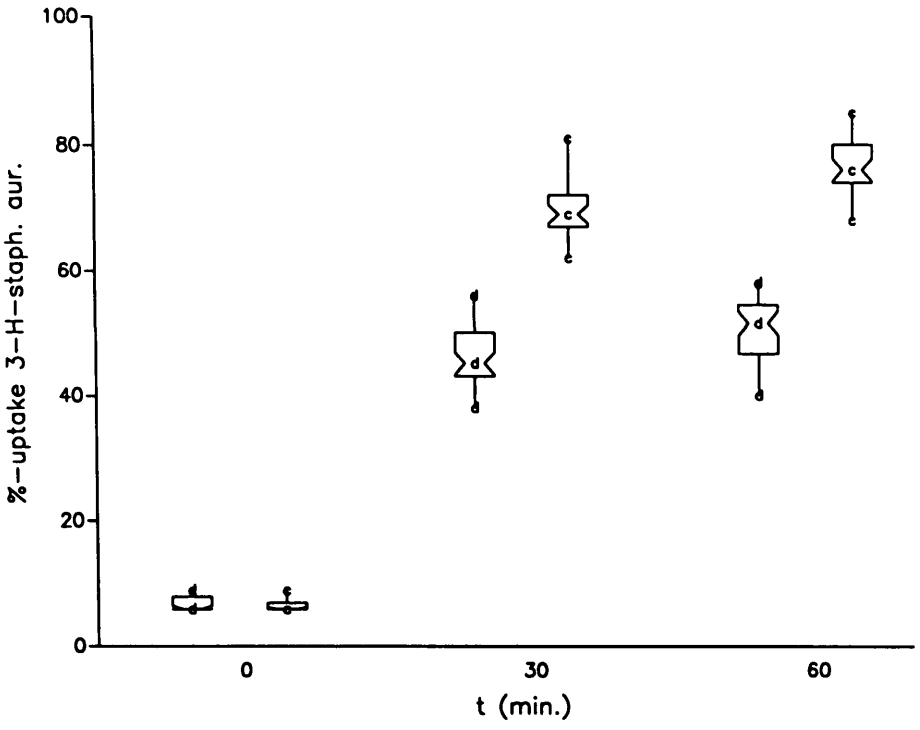

Figure 1—*Polymorphonuclear leukocyte uptake in percentage of ^{3}H-thymidine–labeled Staphylococcus aureus in diabetic patients and nondiabetic control subjects. Data are notched-box plots. C, control; d, diabetes mellitus; t, time. $P < 0.001$ was significant between groups.*

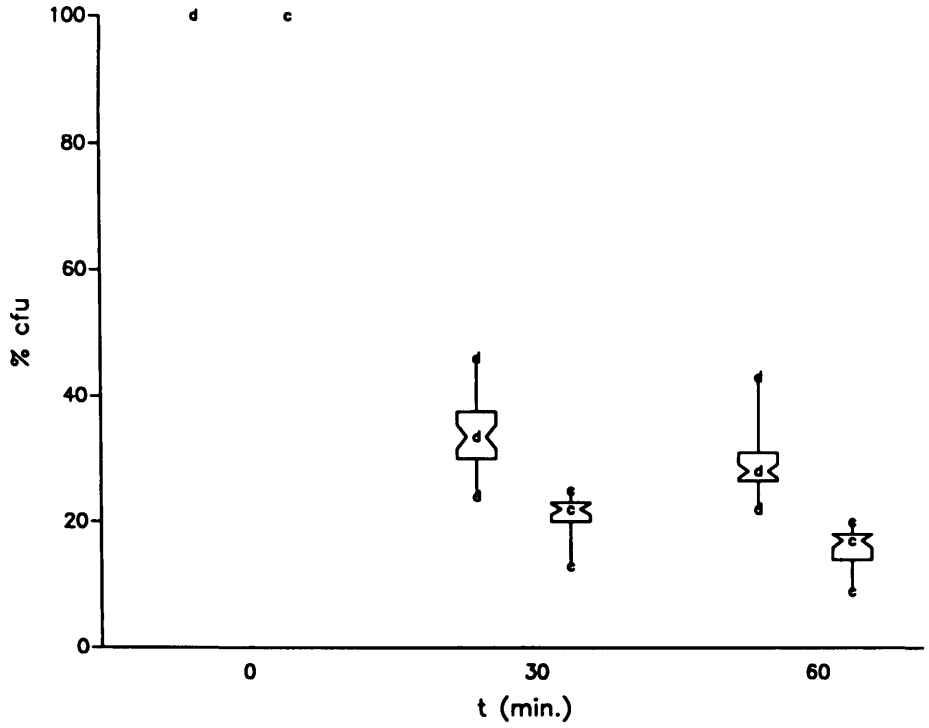

Figure 2—*Percentage of surviving bacteria (colony-forming units [cfu]) in diabetes mellitus (d) and control subjects (c). Data are notched-box plots. t, Time. $P < 0.0001$ was significant between groups.*

measures and simple regression analysis in cooperation with the Institute of Medical Information Sciences (Univ. of Giessen). $P > 0.05$ was nonsignificant.

RESULTS — There was a significant decrease in PMN uptake of ^{3}H-thymidine-labeled *Staphylococcus aureus* in diabetic patients (50.7 ± 4.4%) compared with healthy nondiabetic control subjects (76.8 ± 4.6%, $P < 0.001$; Fig. 1) after 60 min. In bacterial killing, the percentage of surviving bacteria was 29.4 ± 4.2% cfu in the diabetic group compared with 16.3 ± 3.1% cfu in the control group ($P < 0.0001$; Fig. 2).

Chemiluminescence response

In the diabetic and control group PMNs, the resting state showed similar low levels of light emission (data not shown). In response to PMA, PMN from control subjects established a marked increase in light emission, (data not shown). In response to PMA, PMN from control subjects established a marked increase in light emission, peaking at 6 min and declining gradually thereafter. In contrast, PMN from diabetic patients showed a markedly reduced CL response at all time points ($P < 0.001$; Table 2). To evaluate influences of diabetes control on PMN function, linear regression analysis of HbA_1 and glucose levels were conducted. In the diabetic group, PMN ingestion versus HbA_1 showed highly significant negative correlation ($r = -0.67$, $P = 0.001$) and positive correlation for bacterial killing ($r = -0.73$, $P < 0.0001$). This was also true for the CL response, although this did not reach statistical significance ($P = 0.06$). Random blood glucose correlated negatively with PMN ingestion ($r = -0.34$, $P = 0.03$) and CL response ($r = -0.51$, $P = 0.001$) but not with bacteria killing ($P = 0.06$).

CONCLUSIONS — These data demonstrate that PMN ingestion of *staphylococcus aureus* and bacterial killing are significantly reduced in diabetic patients. In addition, we could demonstrate that PMN respiratory burst activity is markedly reduced in diabetic patients and negatively correlated to actual blood glucose concentrations. These data support previous studies that suggest that elevated glucose levels might impair PMN respiratory burst, leading to decreased microbicidal activity (7,8,12). In poorly controlled diabetic patients with ketoacidosis, PMNs reduced phagocytic capacity (5,6). Several disease states or agents that reduce PMN chemiluminescence response have been associated with decreased microbicidal activity and increased susceptibility to infection (7,8,12,13), although the mechanisms for altered cell function remain unclear. Impaired PMN function in diabetes mellitus has been reported to be caused by intracellular sorbitol accumulation (21,22), reduced phosphofructokinase activity (23), or altered arachidonic acid metabolism (24). Taken together, these data on impaired PMN phagocytic function (ingestion, bacteria killing) and decreased chemiluminescence in response to phorbolesters in diabetic patients suggest inhibitory effects of elevated glucose concentrations on PMN function possibly contributing, in part, to altered host defense. However, regardless of the mechanisms involved, further studies including the direct measurements of glycosylation products (25,26) are necessary before the causes of impaired PMN function in diabetes can be clearly defined.

Table 2—*Chemiluminescence response of polymorphonuclear leukocytes from control and diabetic subjects to phorbolmyristic acetate*

CONTROL			
TIME (MIN)			
1	51.7 ± 24.2	(25–130)	45.5
2	184.3 ± 65.4	(110–368)	162.5
3	321.3 ± 87.6	(170–550)	330
4	453.1 ± 114.5	(220–760)	450
5	533.3 ± 127.8	(280–820)	530
6	601.1 ± 164.7	(320–960)	598
15	216.0 ± 102.8	(65–500)	215
30	101.1 ± 47.1	(25–240)	100
60	35.7 ± 22.1	(8–100)	29.5
DIABETIC			
1	24.6 ± 18.9	(4–100)	21
2	101.0 ± 44.4	(11–230)	100
3	170.0 ± 58.6	(26–350)	160
4	229.1 ± 75.7	(70–470)	210
5	250.8 ± 78.2	(99–530)	235.5
6	262.8 ± 85.5	(106–480)	241.5
15	98.7 ± 36.3	(22–170)	100
30	38.5 ± 22.0	(4–76)	36
60	9.8 ± 6.5	(2–25)	9.5

Values are means ± SE with ranges in parentheses. Data represent relative light units ×10.

Acknowledgments— We acknowledge the statistical advice of W. Pabst (Institute of Medical Information Sciences, Univ. of Giessen, Giessen, Germany).

References

1. Larkin JG, Frier BM, Ireland JT: Diabetes mellitus and Infection. *Postgrad Med J* 61:233–37, 1985
2. Murphy DP, Tan JS, File TM: Infectious complications in diabetic patients. *Primary Care* 8:695–714, 1981
3. Rayfield EJ, Ault MJ, Keusch GT, Brothers MJ, Nechemias CH, Smith H: Infection and diabetes: the case for glucose control. *Am J Med* 7:439–50, 1982
4. Wheat LJ: Infection and diabetes mellitus. *Diabetes Care* 3:187–97, 1980
5. Bagdade JD, Root RK, Bulger RJ: Impaired leukocyte function in patients with poorly controlled diabetes. *Diabetes* 23:9–15, 1974
6. Bagdade JD, Stewart M, Walters E: Impaired granulocyte adherence: a reversible defect in host defense in patients with poorly controlled diabetes. *Diabetes* 27:677–81, 1978
7. Shah SV, Wallin JD, Eilen SD: Chemiluminescence and superoxide anion production by leucocytes from diabetic patients. *J Clin Endocrinol Metab* 57:402–409, 1983
8. Nielson CP, Hindson DA: Inhibition of polymorphonuclear leukocyte respiratory burst by elevated glucose concentrations in vitro. *Diabetes* 38:1031–35, 1989
9. Bybee JD, Rogers DE: The phagocytic activity of polymorphonuclear leucocytes obtained from patients with diabetes mellitus. *J Lab Clin Med* 64:1–9, 1964
10. Esman V: The polymorphonuclear leucocyte in diabetes mellitus. *J Clin Chem Clin Biochem* 21:561–67, 1983
11. Tan JS, Anderson JL, Watanakunakorn C, Phair JD: Neutrophil dysfunction in diabetes mellitus. *J Lab Clin Med* 85: 26–30, 1975
12. Nolan CM, Beaty HN, Bagdade JD: Further characterization of impaired bactericidal function of granulocytes in patients with poorly controlled diabetes. *Diabetes* 27:889–94, 1978
13. Repine JE, Clawson CC, Goetz FC: Leucocyte and host defense: bactericidal function of neutrophils from patients with acute bacterial infections and from diabetes. *J Infect Dis* 142:869–75, 1980
14. Dziatkowiak H, Kowalska M, Denys A: Phagocytic and bactericidal activity of granulocytes in diabetic children. *Diabetes* 31:1041–47, 1982
15. Molenaar DM, Palumbo PJ, Wilson WR, Pitts RE Jr: Leukocyte chemotaxis in diabetic patients and their nondiabetic first-degree relatives. *Diabetes* 25:880–83, 1976
16. Mowat AG, Baum J: Chemotaxis of polymorphonuclear leucocytes from patients with diabetes. *N Engl J Med* 24: 621–27, 1971
17. Wilson RM, Reeves WG: Neutrophil phagocytosis and killing in insulin-dependent diabetes. *Clin Exp Immunol* 63: 478–84, 1986
18. Wierusz-Wysocka H, Wysocki H, Siekierka H, Wykretowicz A, Szczepanik A, Klimas R: Evidence of polymorphonuclear neutrophils activation in patients with insulin-dependent diabetes mellitus. *J Leukocyte Biol* 42:519–23, 1987
19. Boyum A: Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab* 21 (Suppl.):77–89, 1968
20. Peterson PK, Verhoef J, Schmeling D, Quie PG: Kinetics of phagocytosis and bacterial killing by human polymorphonuclear leucocytes and monocytes. *J Infect Dis* 136:502–509, 1977
21. Greene DA, Lattmer SA: Altered sorbitol and *myo*-inositol metabolism as the basis for detective protein kinase C and (Na.K)-ATPase regulation in diabetic neuropathy. *Ann NY Acad Sci* 488:334–40, 1986
22. Wilson RM, Tomlinson DR, Reeves WG: Neutrophil sorbitol production impairs oxidative killing in diabetes. *Diabetic Med* 4:37–40, 1987

23. Qvist R, Larkins RG: Decreased stimulated glucose oxidation and iodination by polymorphonuclear leukocytes from insulin-treated diabetic subjects. *Diabetes* 30:256–62, 1981
24. Qvist R, Larkins RG: Diminished production of thromboxane B_2 and prostaglandin E by stimulated polymorphonuclear leukocytes from insulin-treated diabetic subjects. *Diabetes* 32:622–26, 1983
25. Cohan MP: *Diabetes and Protein Glycosylation*. New York, Springer-Verlag, 1986, p. 1–111
26. Brownlee M, Cerami A, Vlassara H: Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med* 318: 1315–21, 1988