RESEARCH NOTE

IMPAIRED INTERLEUKIN-1β EXPRESSION BY MONOCYTES STIMULATED WITH *STAPHYLOCOCCUS AUREUS* IN DIABETES

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Abstract. Diabetic patients with poorly controlled blood glucose have frequent and persistent bacterial infections particularly those infecting the skin, such as *Staphylococcus aureus* and *S. epidermidis*. The function of phagocytes of diabetic patients is believed to be impaired due to hyperglycemia, leading to suboptimal immune response to clear acute infection. The present study investigated interleukin (IL)-1β expression by diabetic patients' monocytes ($n = 22$) experimentally infected with *S. aureus* compared with that from healthy subjects ($n = 30$). In addition, the *in vitro* effect of hyperglycemia on IL-1β expression by monocytes from normal subjects ($n = 18$) stimulated with *S. aureus* and *S. epidermidis* was investigated. Monocytes from diabetic patients, stimulated or not with *S. aureus*, express significantly lower levels of IL-1β than those from healthy subjects. *In vitro* hyperglycemia did not affect IL-1β expression by unstimulated monocytes. However, at the same levels of glucose normal monocytes stimulated with *S. aureus* produce significantly higher IL-1β than those stimulated with *S. epidermidis*. These findings suggest that diabetic patients have abnormally lower IL-1β expression and hyperglycemia is related to abnormal expression of IL-1β by monocytes, which could lead to enhanced susceptibility to infection by the more virulent bacteria.

Keywords: *Staphylococcus aureus*, interleukin-1β, monocytes, diabetes

INTRODUCTION

Non-insulin dependent diabetes mellitus is the most common type of metabolic diseases. Impaired immunity in diabetic patients has long been studied (Bagdade *et al*, 1974; Kjersem *et al*, 1988; Alexiewicz *et al*, 1995; Mazade and Edwards, 2001). This has included immune cells of both innate and adaptive immune system. In diabetes mellitus, skin infection is often associated with an abnormal phagocytic function of macrophages and neutrophils (Bagdade *et al*, 1974; Saiki *et al*, 1980; Jones and

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Peterson, 1981; Glass *et al*, 1986; Kjersem *et al*, 1988; Alexiewicz *et al*, 1995; Mazade and Edwards, 2001). This defect may be due to hyperglycemia (Bagdade *et al*, 1974; Kjersem *et al*, 1988; Alexiewicz *et al*, 1995). Diabetic neutrophils may have reduced superoxide anion production (Noritake *et al*, 1992; Mazade and Edwards, 2001). Hyperglycemia also causes an increase in basal cytosolic calcium concentration in neutrophils (Alexiewicz *et al*, 1995), which may be involved in reduced low-affinity IgG receptor III (FcrRIII) mRNA level (Krol *et al*, 2003). Diabetic macrophages reduce the expression of this lectin-like receptor that recognizes bacterial cell wall resulting in defective intracellular killing (Glass *et al*, 1987; Plotkin *et al*, 1996).

However, findings on defective production of oxidative burst and enzymes by neutrophils and macrophages from diabetic patients have been inconclusive. For instance, one study has shown that superoxide production by neutrophils from diabetic patients is not reduced compared with normal subjects (Lin *et al*, 1993), but another study demonstrated that in diabetic patients neutrophils have reduced hydrogen peroxide level but this is increased in macrophages (Noritake *et al*, 1992). Increased superoxide production in hyperglycemia could be linked to protein kinase C (PKC) activity (Venugopal *et al*, 2002) as hyperglycemia inhibits the function of complement receptor and Fcr receptor-mediated phagocytosis via PKC activation (Saiepour *et al*, 2003). Exposure of neutrophils from normal subjects to high glucose levels causes reduced superoxide production (Perner *et al*, 2003).

Interleukin-1β (IL-1β) is a proinflammatory cytokine that is produced by monocyte lineage cells upon stimulation with bacterial products, including lipopolysaccharide (LPS) endotoxins of gram-negative bacteria, protein exotoxins of gram-positive bacteria, and cell wall glycopeptides, such as teichoic acids and muramyl peptides (Dinarello, 1997). IL-1β also is involved in the production of other inflammatory cytokines including IL-6 and TNF-α, and in the activation of vascular endothelial cells, the increased access of immune effector cells, and the activation of lymphocytes as well as local tissue destruction (Svanborg *et al*, 1999; Miller *et al*, 2006; Sutton *et al*, 2006).

The present study investigated *in vitro* the early innate immune response of monocytes to a common skin infecting gram-positive bacterium, *Staphylococcus aureus*, in diabetes mellitus. Using isolated peripheral blood monocytes from patients with diabetes mellitus and healthy subjects, the production of IL-1β by these cells experimentally infected with *S. aureus* was assessed. In addition, monocytes from healthy subjects were experimentally infected with *S. aureus* and *S. epidermidis* under a hyperglycemic condition and IL-1β production was assessed.

MATERIALS AND METHODS

Subjects

Thirty healthy subjects (35-45 years old) at the Blood Bank Center, Naresuan University Hospital, Thailand and 22 patients with non-insulin dependent diabetes mellitus (35-60 years old) attending the Out-Patient Department of Buddhachinaraj Hospital, Phitsanulok, Thailand. Those were included in the study who had acute infection, either local or systemic, were excluded from the study. All patients included were those having hemoglobin A_{1c} (HbA_{1c}) of more than 5.4%. This study was approved by the Ethics Committee of Naresuan University and written informed consents were obtained from all subjects.

Bacteria

S. aureus and *S. epidermidis* strains were isolated from pus collected from a patient attending Naresuan University Hospital. Identification of bacteria was carried out by conventional biochemical tests (Bascomb and Manafi, 1998). The bacteria were cultured in Trypticase soy broth at 37ºC, harvested at a mid-log phase, and then washed three times in phosphate buffered saline (PBS). Bacterial counts then were measured spectrophotometrically and by viable counting. Prior to use, bacterial solutions were diluted in sterile PBS to achieve the desired concentration.

Isolation of peripheral blood monocytes and experimental infection with bacteria

Fifteen ml of haparinized (20U/ml) venous whole blood were obtained from each subject. Peripheral blood mononuclear cells (PBMC) were isolated by a standard density gradient centrifugation using Lymphoprep (Axis-Shield Poc AS, Oslo, Norway). PBMC were resuspended in complete medium prepared from RPMI-1640 medium (JRH Biosciences, Hampshire, IL) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin (all from PAA Laboratories GmbH, Linz, Austria), and heat inactivated fetal calf serum (FCS) (Gibco, Invitrogen Corporation, New York, NY). The resulting PBMC ($1x10^6$ cells/ml) then were allowed to adhere to 75 cm^3 sterile tissue culture flasks for 1 hour at 37ºC in a 5%CO_2 incubator. The non-adherent cells were removed by washing with complete medium. Adhering monocytes were then harvested by gently shaking the flask and then washing with complete medium. The monocytes were used for experimental infection with bacteria.

Monocytes were infected with *S. aureus* at a multiplicity of infection (MOI) of 20:1 (bacteria:cell) in 24-well plates (Megyeri *et al*, 2002). LPS from *Escherichia coli* (Sigma, St Louis, MO) at a final concentration of 1 µg/ml served as positive control for IL-1β expression by monocytes. Negative controls contained only monocytes. Cultures were incubated at 37ºC in a 5% CO_2 incubator and the reactions were terminated at 2, 4, 6, 8, 16, 24, 36, and 48 hours. The supernatants were collected at each time point and stored at -70ºC until used for analysis of IL-1β. In four cases of healthy donor samples, the monocytes were tested for their survival after being infected with *S. aureus* and *S. epidermidis* for 24 and 48 hours using trypan blue exclusion assay.

For experiments on the effect of glucose levels on monocyte IL-1β expression, monocytes from normal subjects (n = 18) were cultured in complete medium with added glucose at 5 mM (normal level) or at 25 mM (high level) for 48 hours at 37ºC in a 5% CO_2 incubator. Cultures of monocytes without glucose served as negative control. After washing with complete medium, the glucose treated monocytes then were stimulated with either *S. aureus* or *S. epidermidis* at a MOI of 20:1 in 96-well plate for 48 hours. The culture supernatants were collected and stored at -70ºC until used for analysis of IL-1β.

IL-1β ELISA

Culture supernatants were measured in duplicate for IL-1β concentrations using human IL-1β ELISA Kit (BD Biosciences, San Diego, CA) according to the manufacturer's instructions. Absorbance was measured at 450 nm using a Packard SpectraCount® Microplate Photometer (PerkinElmer Life Sciences, Downers Grove, IL).

Proliferation assay

The effect of glucose on the proliferation of monocytes infected with *S. aureus* was assessed. In brief, monocytes from healthy subjects (n = 5) were exposed to glucose from 0 to 35 mM, followed by infection with *S. aureus* at the same MOI. After 48 hours the proliferation was assessed using Cell Proliferation ELISA BrdU (5-bromo-2'-deoxyuridine) colorimetric kit (Roche Applied Science, Indianapolis, IN) according to the manufacture's instructions.

Statistical analysis

Data are presented as mean ± SD. Significant differences between two groups were determined by the two-tailed Student's t-test with $p < 0.05$ as significant using Microsolf Excel Statistical Software.

RESULTS

IL-1β expression by monocytes from diabetic patients stimulated with *S. aureus*

Infection with *S. aureus* or *S. epidermidis* resulted in >92% and >88% of normal monocyte survival after 24 and 48 hours, respectively. At all time points tested the unstimulated monocytes from diabetic patients (n = 22) express significantly lower levels of IL-1β ($p < 0.001$) compared with unstimulated monocytes from normal subjects (n = 30; Fig 1). For monocytes stimulated with *S. aureus*, IL-1β levels are significantly lower in monocytes from diabetic patients ($p < 0.001$) compared with those from normal subjects at all time points tested (Fig 1). In addition, LPS stimulated lower IL-1β expression by monocytes from diabetic patients compared with that of normal subjects, although this is only significant ($p < 0.01$) at 2 to 6 hours.

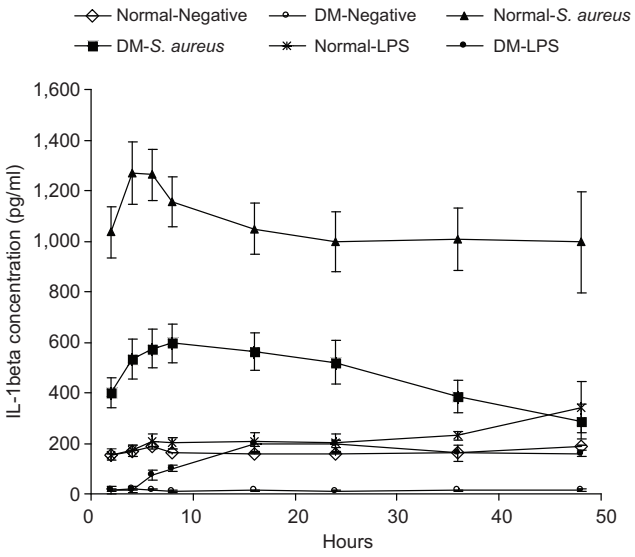

Fig 1–Kinetics of IL-1β expression by monocytes stimulated with *Staphylococcus aurues*. Monocytes from normal healthy subjects (n = 30) and diabetic patients (n = 22) were either stimulated with *S. aureus* (Normal-*S. aureus*; DM-*S. aureus*) or left as negative controls (Normal-Negative; DM-Negative). Stimulation with LPS served as positive control for IL-1β expression (Normal-LPS; DM-LPS). IL-1β level in supernatant was measured using ELISA. Data are shown as mean ± SD.

Effect of glucose levels on monocyte IL-1β expression stimulated with *S. aureus* and *S. epidermidis*

Exposure to normal and high glucose concentrations for 48 hours did not significantly affect IL-1β levels of monocytes from normal subjects (n = 18), stimulated or not stimulated with *S. aureus* (Fig 2). However at the same concentrations of glucose, IL-1β levels of monocytes stimulated with *S. aureus* are significantly higher than those of monocytes stimulated with *S. epidermidis* ($p < 0.05$).

Effect of glucose levels on monocyte proliferation

In order to investigate whether the levels of glucose have any effect on mono-

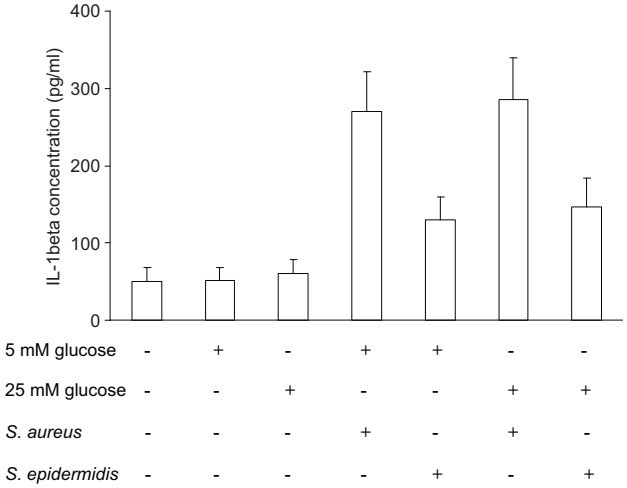

Fig 2–Effect of glucose on IL-1β expression by monocytes stimulated with *S. aureus* or *S. epidermidis*. Monocytes from healthy subjects ($n = 18$) were exposed to 5 or 25 mM glucose or none for 48 hours and then stimulated wtih *S. aurues* or *S. epidermidis* for another 48 hours before IL-1β expression was measured using ELISA. Data are shown as mean ± SD.

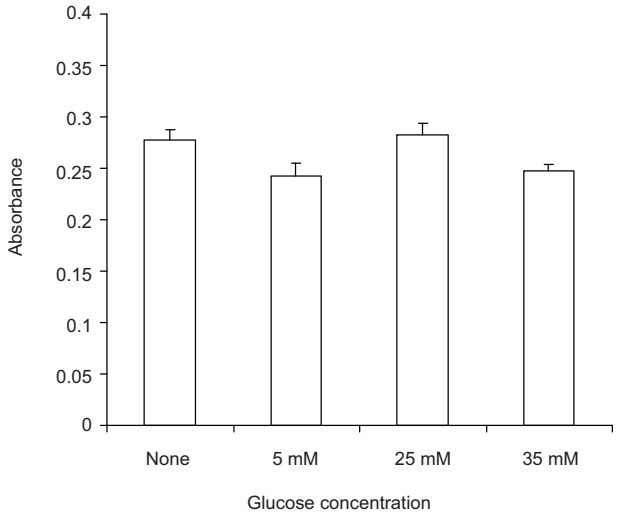

Fig 3–Effect of glucose on monocyte proliferation. Monocytes from healthy subjects ($n = 5$) were exposed to 5 to 35 mM of glucose or none for 48 hours and then cell proliferation was measured using ELISA. Data are shown as mean ± SD of absorbance.

cyte proliferation, monocytes from normal subjects ($n = 5$) were cultured in the presence of different concentrations of glucose. Proliferation of the treated monocytes was similar to that of untreated cells (Fig 3).

DISCUSSION

The present study demonstrated that monocytes from poorly controlled diabetic patients exhibited lower expression of IL-1β levels upon *in vitro* stimulation with a common skin infecting *S. aureus*. The HbA_{1c} of 5.4-10.5% found in these patients corresponded to blood glucose levels of 114-292.5 mg/dl. HbA_{1c} indicates blood glucose level in the previous 120 days. (Goldstein *et al*, 2004) Thus, this finding suggests that hyperglycemia affect the early innate immune response to bacterial infection, indicated by IL-1β expression by monocytes. Although reduced monocyte IL-1β expression from diabetic patients could be due to the death of monocytes after *in vitro* infection, we showed that hyperglycemia did not affect the numbers of monocytes in the cultures. On the other hand, monocytes that had been exposed to high levels of glucose prior to infection may be functionally impaired leading to a decrease in IL-1β expression upon bacterial infection.

The present study also showed that a short exposure to high glucose concentration did not influence the IL-1β levels of monocytes, either resting or stimulated with *S. aureus*. However, at the same concentrations of glucose, IL-1β expression by monocytes was higher when stimu-

lated with *S. aureus* than that stimulated with *S. epidermidis*. *S. aureus* is believed to be more common and virulent pathogenic microorganism than *S. epidermidis*, the latter often infecting immunocompromised host or causing hospital acquired infection (Homer-Vanniasinkam, 2007; Soderquist, 2007; Weisman, 2007; Richadson *et al*, 2008). The present study also demonstrated that high glucose concentrations alone did not affect the number of monocytes. Thus, the high glucose condition as in hyperglycemia may be associated with lower IL-1β response leading to infection with more virulent bacteria.

Other workers have reported an association of hyperglycemia and induction of proinflammatory genes including those encoding IL-1β and TNF-α, which may involve monocyte adhesion, oxidative stress, PKC, as well as p38MAPK controlled by NF-κB (Shanmugam *et al*, 2003). Nevertheless, it has been shown that although hyperglycemia could increase inflammatory cytokine gene expression, the affected cytokines can not be secreted from macrophages as they are inhibited via activation of PKC (Hill *et al*, 1998). The present results, thus, confirm a lower IL-1β secretion by monocytes from diabetic patients and suggest that a higher susceptibility to bacterial infection in diabetes mellitus may be associated with impaired early proinflammatory cytokine response.

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REFERENCES

Alexiewicz JM, Kumar D, Smogorzewski M, Klin M, Massry SG. Polymorphonuclear leukocytes in non-insulin-dependent diabetes mellitus: abnormalities in metabolism and function. *Ann Intern Med* 1995; 123: 919-24.

Bagdade JD, Root RK, Bulger RJ. Impaired leukocyte function in patients with poorly controlled diabetes. *Diabetes* 1974; 23: 9-15.

Bascomb S, Manafi M. Use of enzyme tests in characterization and identification of aerobic and facultatively anaerobic gram-positive cocci. *Clin Microbiol Rev* 1998; 11: 318-40.

Dinarello CA. Interleukin-1. *Cytokine Growth Factor Rev* 1997; 8: 253-65.

Glass EJ, Stewart J, Matthews DM, Collier A, Clarke BF, Weir DM. Impairment of monocyte "lectin-like" receptor activity in type I (insulin-dependent) diabetic patients. *Diabetologia* 1987; 30: 228-31.

Glass EJ, Stewart J, Weir DM. Altered immune function in alloxan-induced diabetes in mice. *Clin Exp Immunol* 1986; 65: 614-21.

Goldstein DE, Little RR, Lorenz RA, Malone IJ, Nathan DM, Peterson CM. Test of glycemia in diabetes. *Diabetes Care* 2004; 27: 1761-73.

Hill JR, Kwon G, Marshall CA, McDaniel ML. Hyperglycemic levels of glucose inhibit interleukin 1 release from RAW 264.7 murine macrophages by activation of protein kinase C. *J Biol Chem* 1998; 273: 3308-13.

Homer-Vanniasinkam S. Surgical site and vascular infections: treatment and prophylaxis. *Int J Infect Dis* 2007; 11(S1): S17-22.

Jones RL, Peterson CM. Hematologic alterations in diabetes mellitus. *Am J Med* 1981; 70: 339-52.

Kjersem H, Hilsted J, Madsbad S, Wandall JH, Johansen KS, Borregaard N. Polymorphonuclear leucocyte dysfunction during short term metabolic changes from normo- to hyperglycemia in type 1 (insulin dependent) diabetic patients. *Infection*

1988; 16: 215-21.

Krol E, Agueel R, Banue S, Smogorzewski M, Kumar D, Massry SG. Amlodipine reverses the elevation in [Ca2+]i and the impairment of phagocytosis in PMNLs of NIDDM patients. *Kidney Int* 2003; 64: 2188-95.

Lin X, Candlish JK, Thai AC. Superoxide production by neutrophils from diabetics and normal subjects in response to glucose and galactose. *Exp Mol Pathol* 1993; 58: 229-36.

Mazade MA, Edwards MS. Impairment of type III group B *Streptococcus*-stimulated superoxide production and opsonophagocytosis by neutrophils in diabetes. *Mol Genet Metab* 2001; 73: 259-67.

Megyeri K, Mandi Y, Degre M, Rosztoczy I. Induction of cytokine production by different staphylococcal strains. *Cytokine* 2002; 19: 206-12.

Miller LS, O'Connel RM, Gutierrez MA, *et al*. MyD88 mediates neutrophil recruitment initiated by IL-1R but not TLR2 activation in immunity against *Staphylococcus aureus*. *Immunity* 2006; 24: 79-91.

Noritake M, Katsura Y, Shinomiya N, *et al*. Intracellular hydrogen peroxide production by peripheral phagocytes from diabetic patients. Dissociation between polymorphonuclear leucocytes and monocytes. *Clin Exp Immunol* 1992; 88: 269-74.

Perner A, Nielsen SE, Rask-Madsen J. High glucose impairs superoxide production from isolated blood neutrophils. *Intensive Care Med* 2003; 29: 642-5.

Plotkin BJ, Paulson D, Chelich A, *et al*. Immune responsiveness in a rat model for type II diabetes (Zucker rat, fa/fa): susceptibility to *Candida albicans* infection and leucocyte function. *J Med Microbiol* 1996; 44: 277-83.

Richadson AR, Libby SJ, Fang FC. A nitric oxide-inducible lactate dehydrogenase enables *Staphylococcus aureus* to resist innate immunity. *Science* 2008; 319: 1672-76.

Saiepour D, Sehlin J, Oldenborg PA. Hyperglycemia-induced protein kinase C activation inhibits phagocytosis of C3b- and immunoglobulin G-opsonized yeast particles in normal human neutrophils. *Exp Diabesity Res* 2003; 4: 125-32.

Saiki O, Negoro S, Tsuyuguchi I, Yamamura Y. Depressed immunological defense mechanisms in mice with experimentally induced diabetes. *Infect Immun* 1980; 28: 127-31.

Shanmugam N, Reddy MA, Guha M, Natarajan R. High glucose-induced expression of proinflammatory cytokine and chemokine genes in monocytic cells. *Diabetes* 2003; 52: 1256-64.

Soderquist B. Surgical site infections in cardiac surgery: microbiology. *APMIS* 2007; 115: 1008-11.

Sutton C, Brereton C, Keogh B, Mills KH, Lavelle EC. A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. *J Exp Med* 2006; 203: 1685-91.

Svanborg C, Godaly G, Hedlund M. Cytokine responses during mucosal infections: role in disease pathogenesis and host defence. *Curr Opin Microbiol* 1999; 2: 99-105.

Venugopal SK, Devaraj S, Yang T, Jialal I. Alpha-tocopherol decrease superoxide anion release in human monocytes under hyperglycemic conditions via inhibition of protein kinase C-alpha. *Diabetes* 2002; 51: 3049-54.

Weisman LE. Antibody for the prevention of neonatal nosocomial staphylococcal infection: a review of the literature. *Arch Pediatr* 2007; 14 (suppl 1): S31-4.