RESEARCH NOTE

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

Sutatip Pongcharoen¹, Wuttichote Chansantor², Kwansuda Supalap³, Poj Jienmongkol⁴ and Pannika Ritvirool Niumsup²

¹Department of Medicine, Faculty of Medicine, ²Department of Microbiology and Parasitology, Faculty of Medical Science, ³Research Unit, Faculty of Medicine, Naresuan University, Phitsanulok; ⁴Internal Medicine Department, Buddhachinnaraj Hospital, Phitsanulok, Thailand

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
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 (Saijeour 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 A1c (HbA1c) 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 37°C, 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 haperinized (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 37°C 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 37°C 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 -70°C 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 37°C 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 -70°C 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.

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-
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-$1\beta$ 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-$1\beta$ expression by monocytes. Although reduced monocyte IL-$1\beta$ 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-$1\beta$ expression upon bacterial infection.

The present study also showed that a short exposure to high glucose concentration did not influence the IL-$1\beta$ levels of monocytes, either resting or stimulated with *S. aureus*. However, at the same concentrations of glucose, IL-$1\beta$ expression by monocytes was higher when stimu-
lated with \textit{S. aureus} than that stimulated with \textit{S. epidermidis}. \textit{S. aureus} is believed to be more common and virulent pathogenic microorganism than \textit{S. epidermidis}, the latter often infecting immunocompromised host or causing hospital acquired infection (Homer-Vanniasinkam, 2007; Soderquist, 2007; Weisman, 2007; Richadson \textit{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 \textit{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 \textit{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


