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High glucose concentration impairs ATP outflow and immunoglobulin production by human peripheral B lymphocytes: Involvement of P2X7 receptor

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ABSTRACT

Aims/hypothesis: Patients with diabetes are more prone to bacterial infections mostly due to hyperglycemia-induced suppression of immune cells function. B lymphocytes by secreting antibodies inhibit microbial replication, but the impact of high glucose concentration on humoral immune response is not fully resolved. The aim of this work was to investigate the effect of high glucose concentration on B cells response to stimulation with a bacterial antigen and autocrine regulation.
Methods: Purified human peripheral blood B cells were cultured at different glucose concentrations and stimulated *in vitro* with *Staphylococus aureus* Cowan I (SAC) plus IL-2. B cells proliferation, differentiation and IgM expression were analyzed by flow cytometry. B cell ATP release and involvement of P2 purinergic receptors in regulation of IgM secretion was assessed.
Results: B cells cultured at 25 mM glucose in response to SAC stimulation released significantly less (~55%) IgM comparing to cells maintained in 5 mM glucose. Under resting and stimulatory conditions B cells released significant quantities of ATP to the culture media, but ATP level decreased when B cells were maintain in high glucose. SAC-induced B cell IgM release was totally blocked by highly selective antagonist (Az11645373) of P2X7 receptor. IgM secretion increased in the presence of potent P2X7 receptor agonist (BzATP), but this effect was abolished by high glucose concentration.
Conclusions/interpretation: High glucose concentration impairs B cell function by suppression of P2X7 receptor-dependent IgM release in response to *in vitro* bacterial antigen stimulation. This alteration may greatly contribute to the impaired humoral immune response in diabetics.

Introduction

Development of diabetes is associated with several specific complications including altered function of immune system. For a long time the association between diabetes mellitus and increased susceptibility to infection lacked strong clinical evidence. However, recent clinical reports provided solid data indicating that susceptibility to infections increases in diabetic patients (Shah and Hux 2003; Boyko et al. 2005; Benfield et al. 2007). Patients with diabetes are generally more prone to certain specific infections, and some occur almost exclusively in them. Diabetes was identified as a risk factor for skin infection, urinary tract infections and for upper and lower respiratory tract infections (Schuetz et al. 2001). Moreover, infections caused by some microorganisms like *Staphylococcus aureus* and *Mycobacterium tuberculosis* occur with increased frequency whereas other pathogens (*Streptococcus pneumonia*, influenza virus) are associated with increased mortality and morbidity (Koziel and Koziel 1995; Kornum et al. 2007). Evidences obtained from animal and *in vitro* studies indicate that diabetic mice experimentally infected with B streptococcal bacteria had reduced clearance of bacteria and higher mortality rates (Edwards and Fuselier 1983). This could be related to the lowered functionality of B cells since impaired humoral immune responses in patients with poor long-term glucose control

Abbreviations: PBL, peripheral blood lymphocytes; SAC, crude extract of *Staphylococus aureus* Cowan I; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PPADS, pyridoxal 5-phosphate 6-azophenyl-2′,4′-disulfonic acid; PPNDS, pyridoxal-5′-phosphate-6-2′-naphthylazo-6′nitro-4′,8′-disulfonate; Az11645373, 3-[1-[[(3′-nitro[1,1′-biphenyl]-4-yl)oxy]methyl]-3-(4-pyridinyl)propyl]-2,4-thiazolidinedione; NF340, 4,4′-(carbonylbis(imin0-31-(4-methylphenylene)carbonylimino))bis-(naphthalene-2,6-disulfonic acid) tetrasodium salt; MRS2279, (1R,2S,4S,5S)-4-[2-chloro6-(methylamino)-9H-purin-9-yl]-2-(phosphonooxyl)bicycle[3.1.0]hexane-1-methanol dihydrogen phosphate ester diamonium salt; BzATP, 2′(3′)-*O*-4-benzoylbenzoyl)-ATP.

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have been also observed (Liberatore et al. 2005; Eibl et al. 2002).

B lymphocytes are responsible for producing the immunoglobulins in response to thymus-dependent/independent antigens. Some subpopulations of B cells (the memory cells) which are responsible for T cell-independent immune response secretes high-affinity IgM in the early phase of infection thereby inhibiting microbial replication in the blood (Shi et al. 2003). The high frequency of infections among diabetics caused by *S. aureus* might result from impaired response of B cells to the bacterial antigens. The lymphocyte dysfunction in diabetes may be attributed to the direct effect of hyperglycemia that alters the regulatory network of immune cells. Adenosine 5′-triphosphate (ATP) and its metabolite adenosine are the core constituents of purinergic signaling network involved in regulation of inflammatory and immune responses (Bours et al. 2006). There is evidence that hypoinsulinemia and hyperglycemia in a cell specific manner significantly affect the metabolism and transport of these purines (Sakowicz-Burkiewicz and Pawelczyk 2011; Rucker et al., 2010). Therefore, the objective of our study was to investigate the impact of high glucose on capacity of human B lymphocytes to produce IgM *in vitro* upon stimulation with *S. aureus* Cowan I (SAC). Since we previously showed that B cells release significant quantities of ATP (Sakowicz-Burkiewicz et al. 2010) the present work was also devoted to examine the high glucose effect on ATP action on human B lymphocyte function.

Methods

Antibodies and reagents

Insulin, penicillin, streptomycin, glucose, crude preparation of inactivated *S. aureus* Cowan I, IL-2, RPMI-1640 medium, Histopaque-1077, Adenosine 5′-triphosphate (ATP) Bioluminescent Assay Kit, alkaline phosphatase-conjugated anti-mouse IgG polyclonal antibody, alkaline phosphatase-conjugated anti-rabbit IgG polyclonal antibody, mouse anti-β-actin monoclonal antibody were obtained from Sigma–Aldrich Sp. z o.o. (Poznan, Poland). Rabbit polyclonal antibody against human P2X7 receptor was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Fluorescein isothiocyanate (FITC)-conjugated mouse anti-human IgM monoclonal antibody, FITC-conjugated mouse anti-human IgGκ monoclonal antibody, phycoerythrin (PE)-conjugated mouse anti-human CD19 monoclonal antibody, PE-conjugated mouse anti-human IgGκ monoclonal antibody were from BD Biosciences Pharmingen (Heilderberg, Germany). FITC-conjugated mouse anti-human CD38 monoclonal antibody was from DAKO (Glostrup, Denmark).

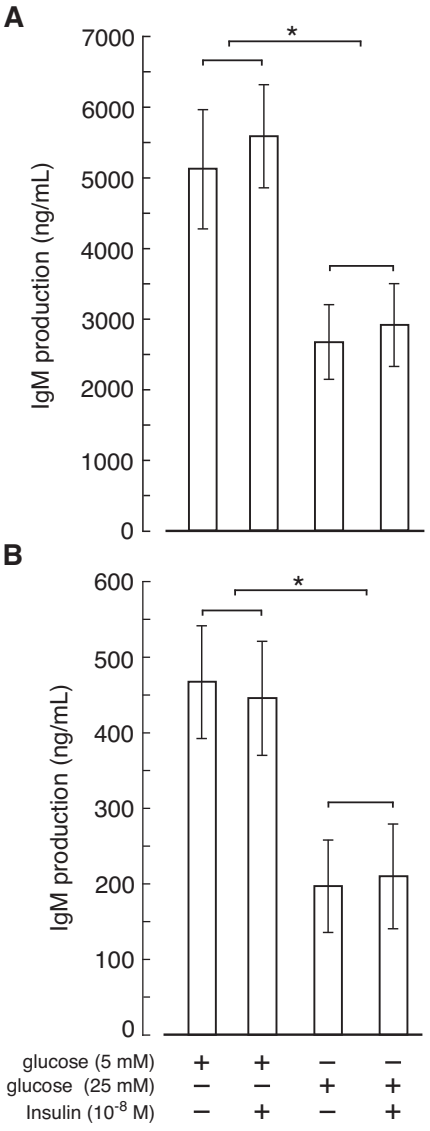

Fig. 1. IgM production by isolated human peripheral blood lymphocytes stimulated *in vitro* with SAC plus IL-2. Isolated lymphocytes (A) or purified B cells (B) were cultured for 48 h in RPMI-1640 medium containing glucose and insulin at indicated concentrations. After 48 h cells were stimulated with SAC plus IL-2 and IgM level in cell culture media was determined on fifth day of culture as described in 'Methods' section. Results are expressed as the mean ± SD of four experiments performed on cells from four different donors. $^{*}P < 0.05$.

Human peripheral blood cells isolation

Fresh buffy coats (not more than 6 h old) were obtained from Regional Blood Bank in Gdańsk. Human peripheral blood lymphocytes were isolated by centrifugation of white blood cells suspension through Histopaque-1077 at 700 g for 30 min at room temperature. Isolated lymphocytes were further purified into B cells by negative selection with magnetic nanoparticles coated with specific monoclonal antibodies (MagCellect Human B cell Isolation Kit) according to manufacturer's protocol. The purity of B cell population was more than 95%.

B cell stimulation

Isolated human peripheral blood lymphocytes or purified B cells were maintained under standard conditions (5% CO_2–95% air, 98% humidity and 37 °C) in RPMI-1640 medium contained glucose and insulin concentration as indicated in the figure legends, supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), and 10% heat-inactivated fetal bovine serum (Gibco). Cells were cultured in flat-bottomed culture bottles (Sarsted) at a density of $\sim 5 \times 10^6$ cells/mL. After 48 h cells were collected and suspended in appropriate medium (low or high glucose). The number of viable cells was determined by Trypan Blue dye exclusion. Only cell cultures with a 95% viability or greater were used. For *in vitro* IgM synthesis 8×10^5 cells (in a volume of 500 μL) were stimulated for 5 days with 0.01% SAC plus 20 U/mL IL-2. Compounds tested were added to the cells (concentrations indicated in the figure legends) 1 h before SAC plus IL-2 stimulation. Control cultures were kept in medium without B cell stimulants. After 120 h supernatants were collected and stored at −20 °C until assayed for IgM content.