JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2012, 63, 6, 661-668
www.jpp.krakow.pl

M. SAKOWICZ-BURKIEWICZ[1], K. KOCBUCH[1], M. GRDEN[1], I. MACIEJEWSKA[2], A. SZUTOWICZ[3], T. PAWELCZYK[1]

IMPACT OF ADENOSINE RECEPTORS ON IMMUNOGLOBULIN PRODUCTION BY HUMAN PERIPHERAL BLOOD B LYMPHOCYTES

[1]Department of Molecular Medicine, Medical University of Gdansk, Gdansk, Poland; [2]Department of Dental Prosthodontics, Medical University of Gdansk, Gdansk, Poland; [3]Department of Laboratory Medicine, Medical University of Gdansk, Gdansk, Poland

Adenosine is an endogenous compound that regulates function of several immune cells including lymphocytes by activating adenosine receptors (ARs). Several reports indicate that stimulation of ARs on lymphocytes affects lymphocyte activation, proliferation and lymphocyte-mediated cytolysis. Unfortunately, most studies focused on T lymphocytes and little information exists on involvement of ARs in B cells regulation. In this study we elucidated the impact of ARs activation on immunoglobulin M (IgM) production by purified human peripheral blood B lymphocytes stimulated *in vitro* with *Staphyloccocus aureus* Covan I (SAC) plus IL-2. Performed experiments showed that endogenous adenosine that is released/produced by human B lymphocytes is able to induce cAMP accumulation in the cell through activation of A_{2A}-AR however, this takes place only when other ARs are inhibited by selective antagonists. We observed that accumulated intracellular cAMP suppressed IgM production by B cells stimulated with SAC plus IL-2. Our experiments showed that human B cells cultured at 25 mM glucose produced significantly less IgM in response to stimulation with SAC comparing to cells maintained in media containing 5 mM glucose. However, the high glucose effect on IgM production by B cells stimulated with SAC depended on other factor/s than ARs.

Key words: adenosine receptors, B lymphocyte, glucose, immunoglobulin M, Staphyloccocus aureus

INTRODUCTION

Adaptive immune response to microbial pathogens depends on the humoral immunity and cell-mediated immunity. The humoral immunity relay on functioning of B lymphocytes that when stimulated with an antigen proliferate and differentiate into cells producing and secreting antibodies. The function of immune cells including B lymphocytes is subjected to regulation by various signaling molecules released by immune and non-immune cells. Some of these molecules are necessary for initiating the immune response, whereas others control the course of action. Adenosine is an endogenous compound that could outflow from the cell or be generated on cell surface from released ATP (1, 2). Its concentration in extracellular space is low, although it might significantly increase under the metabolic stress conditions like diabetes, hypoxia or ischemia (3-5). Its immunomodulatory effects are recognized universally (6, 7). Adenosine elicits its physiological actions by ligation of cell surface P1 purinergic receptors and activation of the downstream intracellular pathways. There are four types of known adenosine receptors namely A_1-AR, A_{2A}-AR, A_{2B}-AR, A_3-AR (8). Adenosine can regulate lymphocyte function indirectly by stimulating AR on macrophages, dendritic cells, neutrophils, mast cells and altering production of several cytokines or directly by binding and activating AR receptors on lymphocytes (7, 9). Several recent reports demonstrated that adenosine activating AR on lymphocytes affects lymphocyte activation, proliferation and lymphocyte-mediated cytolysis (9, 10). Studies on AR-knockout mice showed that activation of A_{2A}-AR on $CD4^+$ T cells suppress the production of IL-2, IFN-γ and IL-4 (11-13). It appears that A_{2A}-AR is the major adenosine receptor influencing the function of lymphocytes. Unfortunately, most if not all of this investigations focused on T lymphocytes and little information exist on involvement of adenosine receptors in regulation of B cells function. Finding a link between adenosine receptors and altered function of B cells could increase our understanding of mechanism/s leading to impaired humoral immune response in pathological conditions like bacteremia related diseases (14, 15) and metabolic diseases like diabetes (16, 17). Development of diabetes is associated with altered adenosine metabolism, transport and ARs expression on B cells (10). Therefore, the goal of our study was to investigate the ARs involvement in regulation of an antigen-induced IgM production by human peripheral blood B lymphocytes and to examine the effect of high glucose concentration on these processes.

MATERIALS AND METHODS

Antibodies and reagents

Insulin, penicillin, streptomycin, crude preparation of inactivated *Staphylococus aureus* Cowan I, IL-2, RPMI-1640 medium, Histopaque-1077, forskolin, adenosine deaminase (ADA), N^6-cyclopentyladenosine (CPA), 2-chloro-N^6-cyclopentyladenosine (CCPA), 1,3-dipropyl-8-cyclopentylxa-

nthine (DPCPX), 2,3-diethyl-4,5-dipropyl-6-phenylpyridine-3-thiocarboxylate-5-carboxylate (ZM1523), N-(4-cyano-phelyl)-2-[4-(2,6-dioxo-1,3-dipropyl-2,3,4,5,6,7-hexahydro-1H-purin-8-yl)-phenoxy]acetamide (MRS 1754), alloxazine, were obtained from Sigma-Aldrich Sp. z o.o. (Poznan, Poland). 5'-N-ethyl-carboxamidoadenosine (NECA), N-[9-chloro-2-(2-furanyl)[1,2,4]-triazolo[1,5-c]quinazolin-5-benzeneacetamide (MRS 1220), 2-[p-(2-carbonyl-ethyl)-phenylethylamino]-5'-N-ethylcarboxamidoadenosine (CGS 21680), 1-butyl-8-(hexahydro-2,5-methanopentalen-3a(1*H*)-yl)-3,7-dihydro-3-(3-hydroxypropyl)-1H-purine-2,6-dione (PSB 36), N^6-(3-iodobenzyl)adenosine-5'-N-methyluronamide (IB-MECA), 2-chloro-N^6-(3-iodobenzyl)adenosine-5'-N-methyluronamide (Cl-IB-MECA), 4-(2-[7-amino-2-[2-furyl]-[1,2,4]triazolo[2,3-α]{1,3,5}triazin-5-yl-amino]ethyl)phenol (ZM 241385), and 8-(3-Chlorostyryl)caffeine (CSC) were from Tocris Bioscence (Nortpoint, UK). Mouse anti-human β-tubulin antibody, goat polyclonal antibodies to A_1-AR, A_{2A}-AR, A_{2B}-AR, A_3-AR antibody, and rabbit anti-goat IgG alkaline phosphatase conjugate were 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 Bioscences Pharmingen (Heilderberg, Germany).

Human peripheral blood cells isolation

Fresh buffy coats (not more than 6 hours old) were obtained from Regional Blood Bank in Gdansk. 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%.

Flow cytometric analysis

Cells were stained in FACS buffer (phosphate buffered saline, 0.5 mM EDTA, 1% FCS) according to standard procedures with anti-CD-19-PE, and anti-IgM-FITC for the surface IgM expression FITC-conjugated or PE-conjugated mouse anti-human IgGκ monoclonal antibody was used as an isotype control. The two-color analysis of B cell surface molecules was performed by a FACScan (Becton Dickinson). The antibody-coated cells were gated on living cells by cell size and granularity and counted by means of flow cytometric analysis.

B cell stimulation

Purified human peripheral blood B lymphocytes were maintained under standard conditions (5% CO_2–95% air, 98% humidity and 37°C) in RPMI-1640 medium contained glucose 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 ~5×10^6 cells/ml. After 48 hours 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 hour before SAC plus IL-2 stimulation. Control cultures were kept in media without B cell stimulants. After 120 hours supernatants were collected and stored at -20°C until assayed for IgM content.

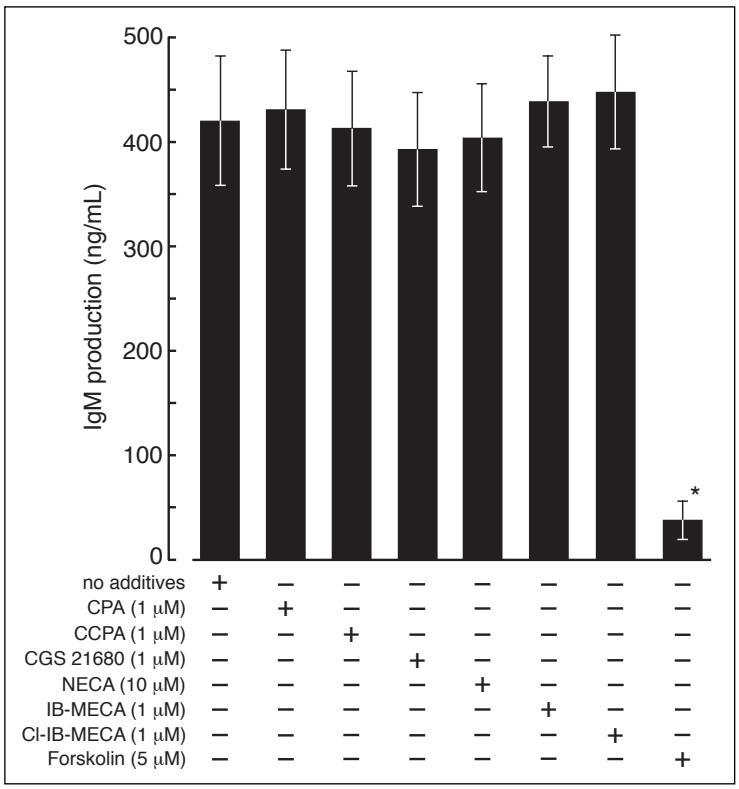

Fig. 1. Effect of adenosine receptors agonists on IgM production by human B cells stimulated *in vitro* with SAC plus IL-2. Purified human peripheral blood B lymphocytes were cultured for 48 hours in RPMI-1640 medium containing 5 mM glucose and stimulated with SAC plus IL-2 as described under "Materials and Methods". The compounds examined were added to the incubation medium one hour before SAC plus IL-2. The A1-AR agonists (CPA, CCPA), the A_{2A}-AR agonist (CGS 21680), the A_3-AR agonists (IB-MECA, Cl-IB-MECA) were used in concentrations 0.01–1 µM. The AR nonselective agonist NECA was used in concentrations 1–10 µM. The bars represent effects of AR agonists evoked by the highest concentration used. On fifth day IgM levels in cell culture media were determined by an ELISA assay as described under "Materials and Methods". The data represent the mean ±S.D. from four experiments on cells from two separate donors. *, $P<0.05$ *vs*. control (no additives).

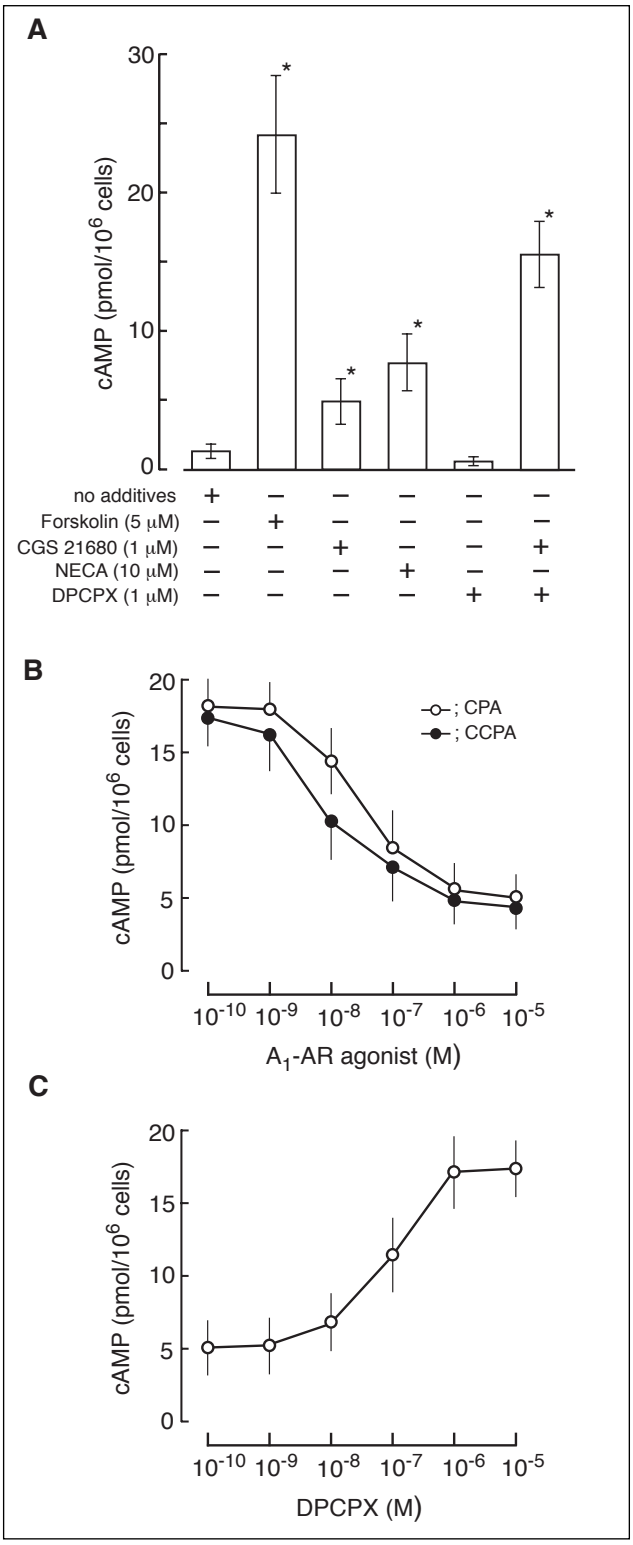

Fig. 2. Accumulation of cAMP in B cells. Purified human peripheral blood B lymphocytes were cultured for 48 hours in RPMI-1640 medium containing 5 mM glucose, exposed to appropriate compounds for 15 min, and the intracellular cAMP level was determined by an ELISA assay as described under "Materials and Methods". (**A**) Changes in the intracellular cAMP level in response to treatment with forskolin or A_{2A}-AR agonist (CGS 21680) in the presence and absence of A_1-AR antagonist (DPCPX), or nonselective AR agonist (NECA). (**B**) Accumulation of cAMP in B cells exposed to increased concentrations of selective A_1-AR agonists in the presence of fixed concentrations (1 μM) of CGS 21680 (A_{2A}-AR agonist) and DPCPX (A_1-AR antagonist). (**C**) Concentration-dependent inhibition of A_1-AR by DPCPX and resulted elevation of cellular cAMP level in B cells incubated in the presence of 1 μM CGS 21680. The data represent the mean ±S.D. from three experiments. *, $P<0.05$ *vs*. control (no additives).

Western blot analysis

The cell extract was obtained as described previously (18). The proteins from cell extracts were separated by 12% SDS-polyacrylamide gel electrophoresis, and electrophoretically transferred to Immobilon poly(vinylidene difluoride) transfer membrane. The membrane was incubated at 4°C (overnight) with 3% BSA in Tris-buffered saline (TBS). The membrane was then cut horizontally at appropriate position (based on position of prestained molecular mass marker) and incubated with appropriate primary antibodies. Next, the membrane strips were washed with TBS and incubated with alkaline phosphatase-conjugated secondary antibodies. Membrane bound antibodies were visualized with 5-bromo-chloro-3-indoylphosphate and nitroblue tetrazolium. The β tubulin was used as a reference protein.

Determination of cAMP

Cells (~2×10^5) were separated from the culture media by centrifugation and the resulting pellet was treated with 0.1 M

HCL. After a 10 min incubation, the lysate obtained was centrifuged and the supernatant was used directly for cAMP determination, according to the manufacturer's protocol for the Direct cAMP Enzyme Immunoassay Kit (Sigma-Aldrich Sp. z o.o., Poznan, Poland).

IgM assay by ELISA

IgM level in cell culture media was determined using enzyme-linked immunosorbent assay (ELISA) kit according to the manufacture's (Dunn Labortechnik GmbH, Asbach, Germany) protocol. The quantity of IgM in the test sample was interpolated from the standard curve and corrected for sample dilution. Results are expressed as nanograms per milliliter of IgM produced in stimulated cultures minus control cultures.

Statistical analysis

Values are reported as means ±S.D. Unpaired Student's *t*-test was applied to assess differences between groups. *P* values below 0.05 were considered as significant.

RESULTS

In order to assess the possible effect of adenosine receptor (AR) activation on IgM production by B cells stimulated *in vitro* with SAC plus IL-2 we cultured highly purified human peripheral blood B lymphocytes with specific AR agonists. Our experiments showed that IgM production by B cells stimulated with SAC was not significantly affected by addition to the incubation medium any of AR agonists used (*Fig. 1*). However, significant decrease of IgM level could be observed when 5 μM forskolin was included in the incubation medium. This indicated that a rise in cAMP level suppress SAC-induced IgM production by B cells. Since, activation of A_{2A}-AR and A_{2B}-AR should result in stimulation of adenylyl cyclase and concomitant increase in intracellular cAMP, we determined cAMP level in B cells exposed to agonists of these receptors. Performed measurements showed only moderate increase of cAMP level in B cells exposed to 1 μM CGS 21680 an agonist of A_{2A}-AR and 10 μM NECA a nonselective AR agonist (*Fig. 2*). However, blockade of A_1-AR by DPCPX (1 μM) significantly altered the CGS 21680-induced raise in cAMP level. Competitive experiments with selective A_1-AR agonists (CPA, CCPA) and A_1-AR antagonist DPCPX confirmed the major role of A_1 receptor in attenuation of CGS 21680-induced accumulation of cAMP in B cells (*Fig. 2*). On the other hand, the relative weak response to A_{2A}-AR stimulation could result from low expression level of this receptor in B lymphocytes comparing to other AR. We have found that the relative expression levels of AR in human B cells (*Fig. 3*) is very similar to that determined in rat B lymphocytes (18, 19). Relatively low specificity and potency of CGS 21680 in humans (20) is another factor that might have an impact on lack of A_{2A}-AR stimulation effect noted in our experiments. Moreover, it has been reported that CGS 21680 binds also to sites unrelated to A_{2A}-AR (21, 22). This means that in cells with few A_{2A}-AR, the effects of CGS 21680 should be analyzed with appropriate skepticism. The relative low potency and selectivity of CGS 21680 binding do not concern A_{2A}-AR antagonists (20), therefore we performed experiments with the use of AR antagonists. To assess the impact of particular AR stimulation on B cell IgM production we blocked all ARs with selective antagonists except one at a time. As can be seen in *Fig. 4*, only stimulation of A_{2A}-AR by endogenous adenosine and simultaneous inhibition of other ARs resulted in suppression of SAC-induced IgM production

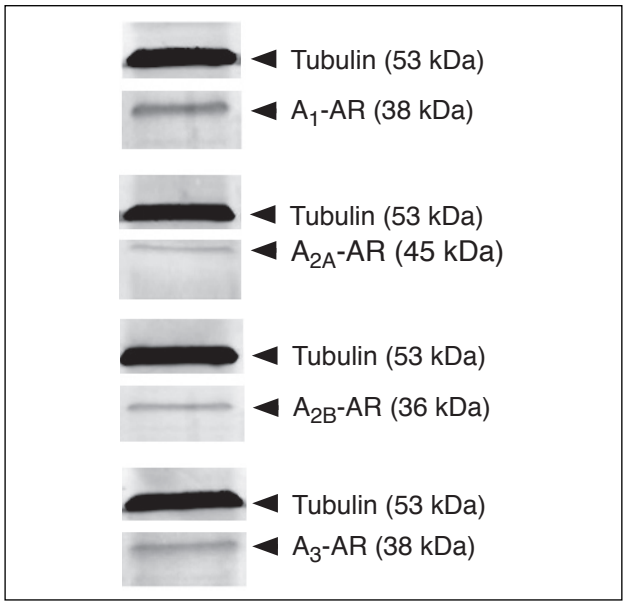

Fig. 3. The protein level of adenosine receptors in human peripheral blood B lymphocytes. The cell extract of purified human peripheral blood B lymphocytes were prepared as described under "Materials and Methods". The proteins were separated on 12% SDS-PAGE and immunoblotted with appropriate antibodies. The blots presented are representative of those obtained in at least three experiments performed on pooled B cells from three separate donors.

by B cells. Addition to the incubation medium adenosine deaminase (0.1 U/ml) restored normal IgM production (*Fig. 4B*). These results also showed that the level of adenosine in B cells culture media was too low to stimulate A_{2B}-AR, which could be activated by addition of 10 μM NECA a nonselective AR agonist (*Fig. 4*). Addition to the incubation media agonists of other ARs (at a time when were not blocked) did not alter the SAC-induced IgM production by B cells (not shown). In the next series of our experiments we tried to answer a question: do the stimulation of A_{2A}-AR by endogenous adenosine have any impact on SAC-induced IgM production by B cells in the presence of other active ARs. The results presented in *Fig. 5* indicate that the presence of active A_1-AR and to the lesser extend A_3-AR prevents A_{2A}-AR-induced decrease of IgM production by B cells stimulated with SAC plus IL-2. It could be assumed that under normal conditions the adenosine receptors on human peripheral blood B lymphocytes remain at the mutual levels in such a fashion, that when stimulated by endogenous adenosine the effect of A_{2A}-AR stimulation is counterbalanced by A_1-AR and A_3-AR activity.

Our previous studies showed that the expression levels of adenosine receptors are significantly changed in several tissues of diabetic animals (3, 23, 24). We observed that exposition of B lymphocytes to high glucose resulted in a significant diminution of A_1-AR, A_{2B}-AR and A_3-AR with no changes in A_{2A}-AR expression (18). Therefore, under high glucose concentration A_{2A}-AR becomes the predominant AR on B cells. Thus, at high glucose concentration B cells might be more sensitive to A_{2A}-AR stimulation. In our current experiments we observed that human B cells cultured at 25 mM glucose produced significantly less IgM in response to stimulation with SAC comparing to cells maintained in media containing 5 mM glucose (*Fig. 6*). However, inclusion of forskolin (5 μM) in the incubation medium resulted in a decrease of IgM to the same level in both high and low

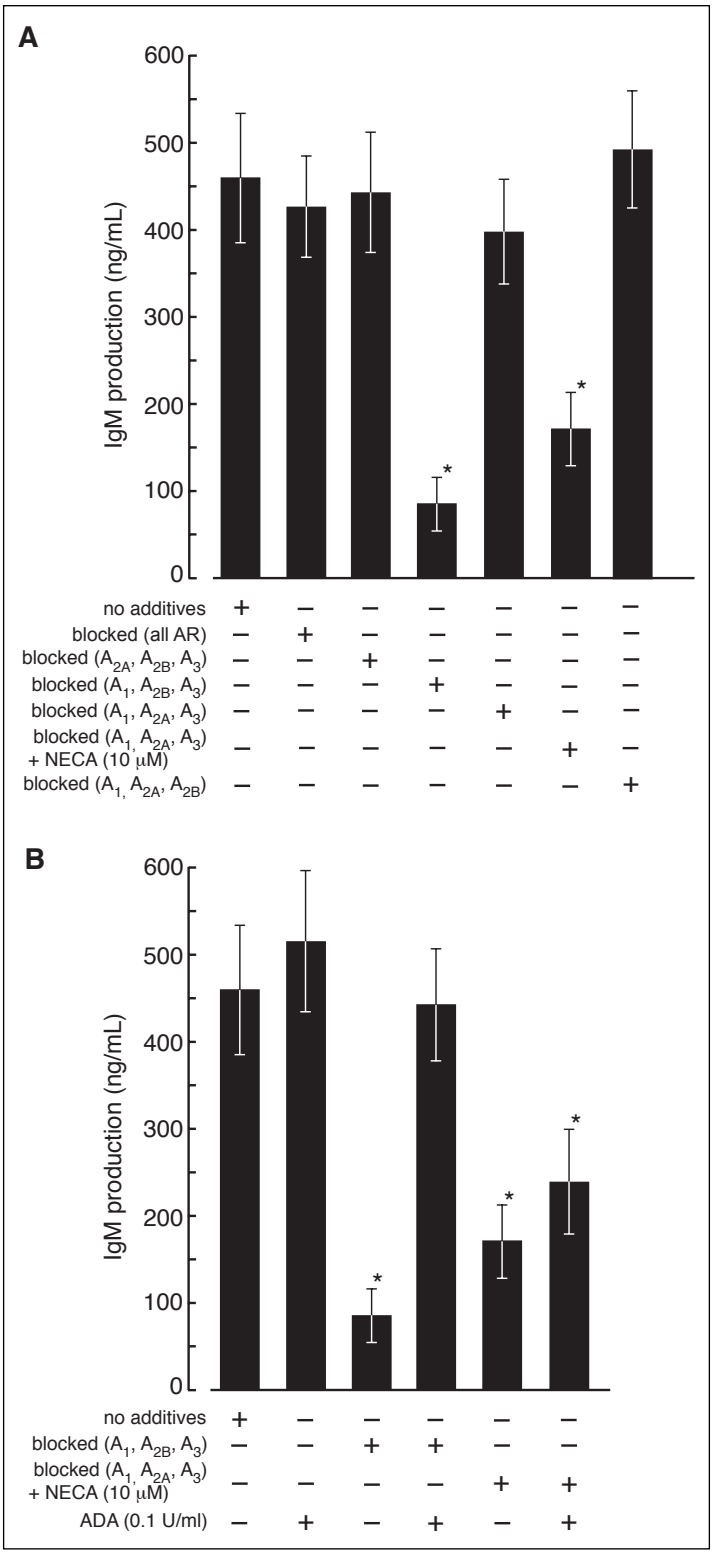

Fig. 4. Effect of adenosine receptor stimulation by endogenous adenosine on IgM production by human B cells stimulated *in vitro* with SAC plus IL-2. Purified human peripheral blood B lymphocytes were cultured for 48 hours in RPMI-1640 medium containing 5 mM glucose and stimulated with SAC plus IL-2 as described under "Materials and Methods". (**A**) One hour before B cells stimulation with SAC plus IL-2, ARs were blocked by combined addition of A_1-AR selective antagonist (DPCPX, 1 μM), A_{2A}-AR selective antagonist (ZM 241385, 1 μM), A_{2B}-AR selective antagonist (MRS 1754, 1 μM), A_3-AR selective antagonist (MRS 1523, 1 μM) as indicated in the figure. On fifth day IgM levels in cell culture media were determined by an ELISA assay as described under "Materials and Methods". Essentially the same results (not shown) were obtained when A_1-AR was blocked with PSB 36 (0.1 μM), A_{2A}-AR was blocked with CSC (1 μM), and A_3-AR was inhibited with MRS 1220 (0.1 μM). The data represent the mean ± S.D. from five experiments on cells from two separate donors. *, $P<0.05$ *vs*. control (no additives). (**B**) Impact of adenosine deaminase (ADA) on SAC-induced IgM production by B cells. One hour before B cells stimulation with SAC plus IL-2, and addition of AR agonist and antagonists to the cell culture media ADA (0.1 U/ml) was added. Next, the activity of ADA in cell culture media was maintained at relatively stable level by supplementation of cell culture media each day with ADA (0.05 U/ml). The data represent the mean ±S.D. from three experiments.

glucose media. This indicated that the cAMP-induced effect/s on IgM production by B cells is not altered by high glucose. Exposition of B cells incubated in 25 mM glucose to selective A_{2A}-AR antagonists (ZM 241385 or CSC), resulted in ~25% increase of SAC-induced IgM production. Although, blockade of A_{2A}-AR with ZM 241385 do not altered the forskolin effect on IgM production (*Fig. 6*). The presence of 1 μM NECA or 1 μM CGS21680 in high glucose media have no effect on SAC-induced IgM production by B cells.

DISCUSSION

Accumulated to date evidence clearly shows crucial role of adenosine receptors in modulation of immune cells function including lymphocytes (25, 26). Most of these studies concerned T lymphocytes with few devoted to B lymphocytes. The pharmacological studies and work with adenosine-receptor-knockout mice revealed that adenosine-induced suppression of T cells function is mediated mainly by A_{2A}-AR (12, 13, 27, 28).

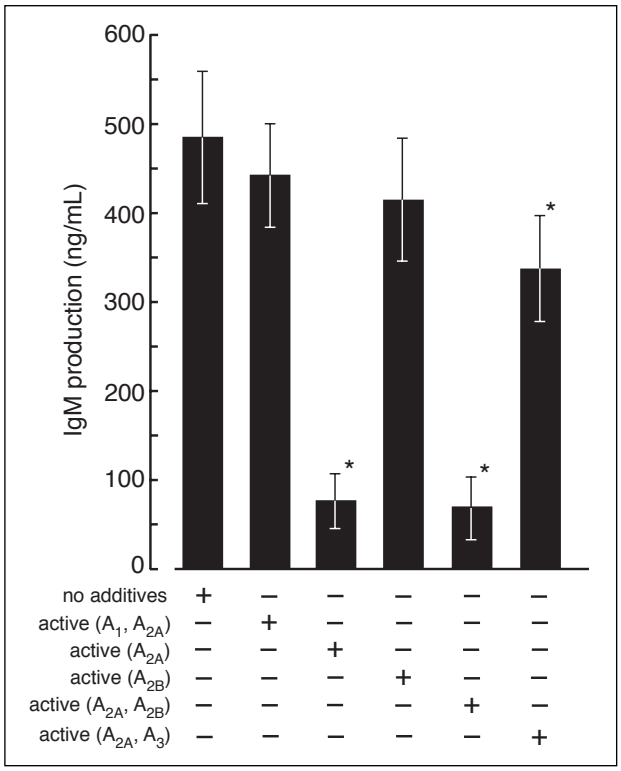

Fig. 5. Impact of A_{2A}-AR stimulation by endogenous adenosine on SAC-induced IgM production by human B cells in the presence of other active adenosine receptor. Purified human peripheral blood B lymphocytes were cultured for 48 hours in RPMI-1640 medium containing 5 mM glucose and stimulated with SAC plus IL-2 as described under "Materials and Methods". One hour before B cells stimulation with SAC plus IL-2, ARs were blocked by addition of A_1-AR selective antagonist (DPCPX, 1 μM), A_{2A}-AR selective antagonist (ZM 241385, 1 μM), A_{2B}-AR selective antagonist (MRS 1754, 1 μM), A_3-AR selective antagonist (MRS 1523, 1 μM) in a fashion that leave active ARs indicated in the *Figure*. On fifth day IgM levels in cell culture media were determined by an ELISA assay as described under "Materials and Methods". The data represent the mean ±S.D. from three experiments. *, P<0.05 *vs*. control (no additives).

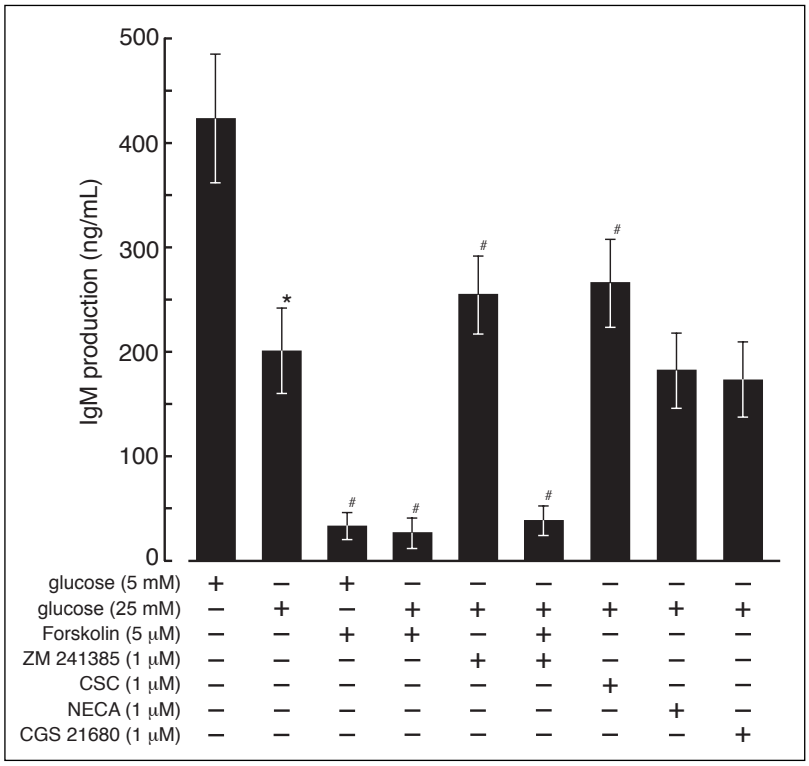

Fig. 6. Effect of glucose concentration and A_{2A}-AR on IgM production by human B cells stimulated *in vitro* with SAC plus IL-2. Purified human peripheral blood B lymphocytes were cultured for 48 hours in RPMI-1640 medium containing 5 mM or 25 mM glucose concentration and stimulated with SAC plus IL-2 as described under "Materials and Methods". Forskolin, A_{2A}-AR selective antagonists (ZM 241385, CSC), nonselective AR agonist (NECA), or A_{2A}-AR agonist (CGS 21680) were added one hour before B cells stimulation with SAC plus IL-2. On fifth day IgM levels in cell culture media were determined by an ELISA assay as described under "Materials and Methods". The data represent the mean ±S.D. from five experiments on cells from two separate donors. On cells from each donor parallel incubations in 5 and 25 mM glucose were performed. *, P<0.05 *vs*. 5 mM glucose; #, P<0.05 *vs*. 25 mM glucose alone.

Investigations performed on chicken B cell lymphoma DT40 and murine splenic B cells showed that adenosine blocked BCR-induced NF-κB activation trough the engagement of the A_2 adenosine receptors (29). The ability of exogenous adenosine to induction cAMP accumulation was also reported in B lymphocytes isolated from patients with chronic lymphocytic leukemia (30). This corresponds with our results presented in this contribution; however, for the first time we documented that the adenosine-induced cAMP alters the IgM production by human peripheral blood B cell stimulated with a bacterial antigen. We showed that endogenous adenosine that is released/produced by normal human B lymphocytes is able to induce cAMP accumulation through activation of A_{2A}-AR however, this takes place only when other ARs are inhibited by selective antagonists. This means that under normal conditions stimulation of A_{2A}-AR on B cells by endogenous adenosine is

counterbalanced by simultaneous activation of other ARs namely A_1-AR and A_3-AR.

It could be assumed that conditions changing the expression level of ARs in a way that alters their mutual levels should also result in altered response of the cell to adenosine. Such a situation takes place in diabetes when the expression level of adenosine receptors changes in several tissues including B lymphocytes (3, 10, 23, 24). Studies performed on animals showed that insulin and glucose levels are the major factors responsible for altered ARs expression (18, 19). In humans, clinical observations indicate that development of diabetes is associated with impairment of humoral immunity. 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 (31). Moreover, infections caused by some microorganisms like *Staphylococcus aureus* and *Mycobacterium tuberculosis* occur with increased frequency whereas other pathogens (*Streptococcus pneumonia*, influenzavirus) are associated with increased mortality and morbidity (32, 33). 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 (34). This could be related to the lowered functionality of B cells since impaired humoral immune responses in patients with poor long-term glucose control have been also observed (35, 36). This assumption is in line with our observations indicating that IgM production by B lymphocytes isolated from diabetic rats and stimulated with a bacterial antigen is lowered comparing to B cells from healthy animals (unpublished). Similar observations were reported from study on streptozotocin-induced diabetic mice (37). Results presented in this report show that high concentration of glucose impairs the IgM production by human B cells stimulated with a bacterial antigen. It could be assumed that this is the result of high glucose-induced changes in ARs expression on B cells in such a way that A_{2A}-AR becomes the major adenosine receptor. However, inhibition of A_{2A}-AR by a selective antagonist resulted only in marginal increase of IgM production. This means that altered response to SAC stimulation of B cells cultured in high glucose is caused by other factor than changes in the expression level of ARs. Our recent studies showed that P2 purinoceptors, are involved in regulation of IgM production by human B lymphocytes (38).

Concluding, presented evidence indicates that at low glucose concentration B cell IgM production in response to a bacterial antigen stimulation is maintained by cooperative action of all ARs and could be altered by pharmacological manipulation of adenosine receptors activities and cAMP accumulation level. We assume that high glucose-induced decrease of B cell immunoglobulin production depends on other factors than altered activity of ARs.

Acknowledgements: This work was supported by the Polish Ministry of Science and Higher Education grant no. 2P05A 081 29 to TP.

Conflict of interests: None declared.

REFERENCES

1. Sakowicz M, Szutowicz A, Pawelczyk T. Differential effect of insulin and elevated glucose level on adenosine transport in rat B lymphocytes. *Int Immunol* 2005; 17: 145-154.
2. Sakowicz-Burkiewicz M, Kocbuch K, Grden M, Szutowicz A, Pawelczyk T. Adenosine 5'-triphosphate is the predominant source of peripheral adenosine in human B lymphoblast. *J Physiol Pharmacol* 2010; 61: 491-499.
3. Pawelczyk T, Grden M, Rzepko R, Sakowicz M, Szutowicz A. Region-specific alterations of adenosine receptors expression level in kidney of diabetic rat. *Am J Pathol* 2005; 167: 315-325.
4. Deussen A, Stappert M, Schafer S, Kelm M. Quantification of extracellular and intracellular adenosine production: understanding the transmembraneus concentration gradient. *Circulation* 1999; 99: 2041-2047.
5. Sitkovsky MV, Lukashev D. Regulation of immune cells by local-tissue oxygen tension: HIF1 alpha and adenosine receptors. *Nat Rev Immunol* 2005; 5: 712-721.
6. Hasko G, Cronstein BN. Adenosine: an endogenous regulator of innate immunity. *Trends Immunol* 2004; 25: 33-39.
7. Bours MJ, Swennen EL, Virgilio F, Cronstein BN, Dagnelie PC. Adenosine 5'-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. *Pharmacol Ther* 2006; 112: 358-404.
8. Fredholm BB, Ijzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol Rev* 2001; 53: 527-552.
9. Hasko G, Linden J, Cronstein B, Pacher P. Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. *Nat Rev* 2008; 7: 759-770.
10. Sakowicz-Burkiewicz M, Pawelczyk T. Recent advances in understanding the relationship between adenosine metabolism and the function of T and B lymphocytes in diabetes. *J Physiol Pharmacol* 2011; 62: 505-512.
11. Naganuma M, Wiznerowicz EB, Lappas CM, Linden J, Worthington MT, Ernst PB. Cutting edge: critical role for A2A adenosine receptors in the T cell-mediated regulation of colitis. *J Immunol* 2006; 177: 2765-2769.
12. Sevigny CP, Li L, Awad AS, *et al.* Activation of adenosine 2A receptors attenuates allograft rejection and alloantigen recognition. *J Immunol* 2007; 178: 4240-4249.
13. Lappas CM, Rieger JM, Linden J. A_{2A} adenosine receptor induction inhibits IFN-γ production in murine $CD4^+$ T cells. *J Immunol* 2005; 174: 1073-1080.
14. Schuetz P, Castro P, Shapiro NI. Diabetes and sepsis: Preclinical findings and clinical relevance. *Diabetes Care* 2011; 34: 771-778.
15. Grebowska A, Moran AP, Bielanski W, *et al.* Helicobacter pylori lipopolysaccharide activity in human peripheral blood mononuclear leukocyte cultures. *J Physiol Pharmacol* 2010; 61: 437-442.
16. Wender-Ozegowska E, Zawiejska A, Michalowska-Wender G, Iciek R, Wender M, Brazert J. Metabolic syndrome in type 1 diabetes mellitus. Does it have impact on the course of pregnancy? *J Physiol Pharmacol* 2011; 62: 567-573.
17. Joshi M, Caputo GM, Weitekamp MR, Karchmer AW. Infections in patients with diabetes mellitus. *N Engl J Med* 1999; 341: 1906-1912.
18. Sakowicz-Burkiewicz M, Kocbuch K, Grden M, Szutowicz A, Pawelczyk T. Protein kinase C mediated high glucose effect on adenosine receptors expression in rat B lymphocytes. *J Physiol Pharmacol* 2009; 61: 491-499.
19. Sakowicz-Burkiewicz M, Kocbuch K, Grden M, Szutowicz A, Pawelczyk T. Regulation of adenosine receptors expression in rat B lymphocytes by insulin. *J Cell Biochem* 2010; 109: 396-405.
20. Kull B, Arslan G, Nilsson C, *et al.* Differences in the order potency for agonists, but not antagonists, at human and rat adenosine A_{2A} receptors. *Biochem Pharmacol* 1999; 57: 65-75.
21. Cunha RA, Johansson B, Constantino MD, Sebastiao AM, Fredholm BB. Evidences for high-affinity sites for the

adenosine A_{2A} receptor agonist [^{3}H]CGS 21680 in the hippocampus and cerebral cortex that are different from striatal A_{2A} receptors. *Naunyn Schmiedebergs Arch Pharmacol* 1996; 353: 261-271.
22. Lindstrom K, Ongini E, Fredholm BB. The selective adenosine A_{2A} receptor antagonist SCH 58261 discriminates between two different binding sites for [^{3}H]CGS 21680 in the rat brain. *Naunyn Schmiedebergs Arch Pharmacol* 1996; 354: 539-541.
23. Grden M, Pogorska M, Szutowicz A, Pawelczyk T. Altered expression of adenosine receptors in heart of diabetic rat. *J Physiol Pharmacol* 2005; 56: 587-597.
24. Grden M, Podgorska M, Szutowicz A, Pawelczyk T. Diabetes-induced alterations of adenosine receptors expression level in rat liver. *Exp Mol Pathol* 2007; 83: 392-398.
25. Gessi S, Varani K, Merighi S, *et al.* Adenosine and lymphocyte regulation. *Purinergic Signal* 2007; 3: 109-116.
26. Ernst PE, Garrison JC, Thompson LF. Much Ado about adenosine: adenosine synthesis and function in regulatory T cell biology. *J Immunol* 2010; 185: 1993-1998.
27. Sakowicz-Burkiewicz M, Kocbuch K, Grden M, Szutowicz A, Pawelczyk T. Diabetes-induced decrease of adenosine kinase expression impairs the proliferation potential of diabetic rat T lymphocytes. *Immunology* 2006; 118: 402-412.
28. Pawelczyk T, Sakowicz-Burkiewicz M, Kocbuch K, Szutowicz A. Differential effect of insulin and elevated glucose level on adenosine handling in rat T lymphocytes. *J Cell Biochem* 2005; 96: 1296-1310.
29. Minhuet S, Huber M, Resenkranz L, Schamel WWA, Reth M, Brummer T. Adenosine and cAMP are potent inhibitors of the NF-κB pathway downstream of immunoreceptors. *Eur J Immunol* 2005; 35: 31-41.
30. Conigrave AD, Fernando KC, Gu B, *et al.* P2Y11 receptor expression by human lymphocytes: evidence for two cAMP-linked purinoceptors. *Eur J Pharmacol* 2001; 426: 157-163.
31. Schuetz P, Castro P, Shapiro NI. Diabetes and sepsis: preclinical findings and clinical relevance. *Diabetes Care* 2001; 34: 771-778.
32. Koziel H, Koziel MJ. Pulmonary complications of diabetes mellitus. Pneumonia. *Infect Dis Clin North Am* 1995; 9: 65-96.
33. Kornum JB, Thomsen RW, Riis A, Lervang HH, Schonheyder HC, Sorensen HT. Type 2 diabetes and pneumonia outcomes: a population-based cohort study. *Diabetes Care* 2007; 30: 2251-2257.
34. Edwards MS, Fuselier PA. Enhanced susceptibility of mice with streptozotocin-induced diabetes to type II group B streptococcal infection. *Infect Immun* 1983; 39: 580-585.
35. Eibl N, Spatz M, Fischer GF, *et al.* Impaired primary immune response in type-1 diabetes: results from a controlled vaccination study. *Clin Immunol* 2002; 103: 249-259.
36. Liberatore RR, Barbosa SF, Alkimin MG, *et al.* Is immunity in diabetic patients influencing the susceptibility to infections? Immunoglobulins, complement and phagocytic function in children and adolescents with type 1 diabetes mellitus. *Pediatr Diabetes* 2005; 6: 206-212.
37. Rubinstein R, Genaro AM, Motta A, Cremachi G, Wald MR. Impaired immune responses in streptozotocin-induced type I diabetes in mice. Involvement of high glucose. *Clin Exp Immunol* 2008; 154: 235-246.
38. Sakowicz-Burkiewicz M, Kocbuch K, Grden M, Maciejewska I, Szutowicz A, Pawelczyk T. High glucose concentration impairs ATP outflow and immunoglobulin production by human peripheral B lymphocytes: involvement of P2X7 receptor. *Immunobiology* 2012; July 21: [epub ahead of print].

Received: August 6, 2012
Accepted: December 10, 2012

Author's address: Prof. Tadeusz Pawelczyk, Department of Molecular Medicine, Medical University of Gdansk, 7 Debinki Street, paw. 29, 80-211 Gdansk, Poland
E-mail: tkpaw@amg.gda.pl