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Review

# Purinergic signaling in B cells

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**Adenosine and adenosine triphosphate are involved in purinergic signaling which plays an important role in control of the immune system. Much data have been obtained regarding impact of purinergic signaling on dendritic cells, macrophages, monocytes and T lymphocytes, however less attention has been paid to purinergic regulation of B cells. This review summarizes present knowledge on ATP- and Ado-dependent signaling in B lymphocytes. Human B cells have been shown to express $A_1$-AR, $A_{2A}$-AR, $A_{2B}$-AR and $A_3$-AR and each subtype of P2 receptors. Surface of B cells exhibits two antagonistic ectoenzymatic pathways, one relies on constitutive secretion and resynthesis of ATP, while the second one depends on degradation of adenosine nucleotides to nucleosides and their subsequent degradation. Inactivated B cells remain under the suppressive impact of autocrine and paracrine Ado, whereas activated B lymphocytes increase ATP release and production. ATP protects B cells from Ado-induced suppression and exerts pro-inflammatory effect on the target tissues, and it is also involved in the IgM release. On the other hand, Ado synthesis is necessary for optimal development, implantation and maintenance of the plasmocyte population in bone marrow in the course of the primary immune response. Moreover, Ado plays an important role in immunoglobulin class switching, which is a key mechanism of humoral immune response. Disruption of purinergic signaling leads to severe disorders. Impairment of Ado metabolism is one of the factors responsible for common variable immunodeficiency. There are several lines of evidence that dysfunction of the immune system observed during diabetes may in part depend on disrupted ATP and Ado metabolism in the B cells.**

**Key words**: B cells, purinergic signaling, adenosine, adenosine triphosphate, P1 receptors, P2 receptors

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**Abbreviations**: Ado, adenosine; AC, adenylate cyclase; ADA, adenosine deaminase; ADP, adenosine diphosphate; ADK, ecto-adenylate kinase; AMP, adenosine monophosphate; AK, adenosine kinase; ATP, adenosine triphosphate; BCR, B-cell receptors; Breg, regulatory B cells; cAMP, cyclic adenosine monophosphate; CNT, sodium cation dependent concentrative nucleoside transporters; CVID, common variable immunodeficiency; EBV, Epstein Barr virus; ENT, equilibrative nucleoside transporters; ERK1, extracellular signal-regulated kinases; FO B cells, follicular B cells; GC, germinal center; ICS, immunoglobulin class switching; Ino, inosine; CD62L, L-selectin; 5′-NT, CD73, ecto-5′-nucleotidase; NDP, ecto-NDP-kinase; NTPDase1, ectonucleoside triphosphate diphosphohydrolase-1; MHC, major histocompatibility complex; MZ, marginal zone; MMPs, matrix metalloproteinases; MAPK, mitogen activated protein kinase; NTPs, nucleoside triphosphates; PKC, protein kinase C; Tc, cytotoxic T cells; Teff, effector T cells; Treg, regulatory T cells; TCR, T cell receptor; TI, T cell-independent; TLR, toll-like receptor; TF, transcription factor; $T_{FH}$, follicular helper T cells; Th, helper T cells

## PURINERGIC SIGNALING

Adenosine triphosphate (ATP) and its breakdown products, mainly adenosine diphosphate (ADP) and adenosine (Ado), are ligands for purinergic receptors and function as extracellular messengers. Purinergic signaling plays an important role in many biological processes, including: exocrine and endocrine secretion, immunological responses, aggregation of platelets, vasodilatation and cellular proliferation, differentiation and apoptosis (Burnstock, 2007). Purinergic network consist of two types receptors, namely P1 and P2. P1 receptors selectively bind Ado and subdivide into $A_1$-AR, $A_{2A}$-AR, $A_{2B}$-AR and $A_3$-AR subtypes. These receptors belong to the rhodopsin-like family of G protein-coupled receptors and interact with adenylate cyclase (AC). $A_1$-AR and $A_3$-AR negatively affect AC *via* α subunits of $G_{i/o}$ protein, and thus inhibiting cyclic adenosine monophosphate (cAMP) synthesis, while $A_{2A}$-AR and $A_{2B}$-AR stimulate AC by $G_s$ and promote cAMP accumulation. P2 receptors are activated by ATP and its derivatives, and consist of two types: P2X and P2Y. P2X receptors are ligand-gated ion channels and to date 7 subunits ranging from $P2X_1$ to $P2X_7$ have been identified. Molecular structure of a single P2X subunit consists of intracellularly located N- and C-termini with consensus binding motifs for protein kinases, two transmembrane-spanning regions (TM1, TM2) responsible for channel gating and lining the ion pore, a large cysteine-rich extracellular loop forming disulfide bridges, a hydrophobic H5 region responsive to channel modulation by cations, and ATP-binding site. The subunits couple into functional homotrimers or heterotrimers, while trimers are capable to form hexamers. Family of P2Y receptors consists of 8 receptor subtypes, namely $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$. Each subunit possesses an extracellular N-terminus and intracellular C-terminus reach in kinase binding motifs. Seven spanning regions lay within the transmembrane domain and are involved in forming of a ligand docking pocket. Intracellular loops and the C-terminus display structural diversity, which determines the degree of coupling with G proteins. Each P2Y receptor connects to a single G protein heterotrimer, typically to $G_{q/11}$. $P2Y_{11}$ is able to interact with $G_{q/11}$ as well as with $G_s$ and $P2Y_{12\text{-}14}$ coupled to $G_i$. Depending on conditions, P2Y form homomultimers or heteromultimers (Burnstock, 2007).

## LYMPHOCYTES IN THE IMMUNE SYSTEM

Human immune system acts through two different types of responses acting in close cooperation. Immediate, innate response comprises myeloid lineage derived cells and lymphocyte natural killer (NK) cells derived from lymphoid progenitors. Constitutive innate response is independent of the count of previous infections and depends only on the type of the inducing factor and remains similar during one's life time. Extent of the delayed acquired (adaptive) response is increased by recurrent contacts with stimulatory agents due to the phenomenon of immune memory and is carried out by cells of the lymphoid lineage (various types of T and B lymphocytes), as well as the myeloid accessory cells. For activation, the T lymphocyte requires presentation of a processed antigen by professional antigen presenting cells (dendritic cells, B-cells, or macrophages), together with the major histocompatibility complex (MHC) type I or II (Chaplin, 2010).

Three major types of T lymphocytes are recognized: Th (helper) $CD4^+$, Tc $CD8^+$ (cytotoxic) and Treg (regulatory). The major feature of a T lymphocyte is expression on its surface of the αβ T cell antigen receptor (TCR). Naive $CD4^+$ T cells may transform towards Th1, Th2, and Th17 subtypes depending on the type of cytokines released into the microenvironment during activation. Th1 cells express transcription factor (TF) T-bet and produce IL-2, IFN-γ, and lymphotoxin. Th2 cells express TF GATA-3 and release IL-4, IL-5, IL-9, IL-13, and GM-CSF. Th17 cells are marked by TF RORC2 expression and production of IL-6 and IL-17. It is generally approved that Th1 cells promote cellular immune responses, whereas Th2 are involved in humoral mechanisms. Subpopulation of circulating $CD4^+$ T lymphocytes gives rise to regulatory T cells (Tregs), which are involved in modulation of the immune response. This fraction can be divided into natural and induced Tregs. Natural Tregs express surface CD4 and CD25 together with a nuclear forkhead box P3 (Foxp3) TF and secrete immunosuppressive cytokines, like TGFβ and IL-10. Induced Tregs express Foxp3 variably and their major product is IL-10 (Chaplin, 2010).

B cells exhibit expression of the membrane B cell receptor (BCR), which can recognize antigens in their native forms, thus B lymphocytes do not need antigen presentation for activation. Antigen recognition, together with signals from activated Th2 cells, induces B cells to proliferate and generate effector plasma cells and memory B cells (Chaplin, 2010). B cells may also undergo activation in a T cell-independent manner by T cell-independent (TI) antigens (Herlands *et al.*, 2008). TI antigens consist of foreign polysaccharides and unmethylated CpG DNA, and can induce rapid and less specific humoral response when compared to the T cell-dependent activation (Nutt *et al.*, 2015).

The B cells may be subdivided into separate populations depending on their maturity and function. B cell lineages arise directly from common lymphoid 2 progenitor (LCA-2) cells (Tobòn *et al.*, 2013). Bone marrow cells produce signals (cytokines, TFs) which induce differentiation of LCA-2 cells to pre-B cells. Mature B lymphocytes bear only one type of specific BCR, thus the generation of the receptor is a critical step in development of B cells (Tobòn *et al.*, 2013). Immature B cells with already developed BCR migrate from bone marrow to circulation as transitional B cells (Palanichamy *et al.*, 2009). Transitional B cells are characterized by surface expression of CD20, CD38, CD24, BR3 and IgM and nuclear presence of TFs, such as Pax5, EBF, E2A and Oct2 (Melchers, 2015). Current criteria recognize three groups of B cells, i.e. T1, T2 and T3 cells (Palanichamy *et al.*, 2009). Maturation of transitional B cells may occur in the spleen and lymphoidal node follicles, resulting in generation of follicular (FO), marginal zone (MZ), germinal center (GC), and memory B cells (Chung *et al.*, 2002; Melchers, 2015). MZ B cells are non-circulating and reside in the marginal zone of the spleen. They are involved in the early adaptive immune responses since the marginal zones intake high volumes of blood from general circulation (Pillai *et al.*, 2005). Phenotype of MZ B cells consists of surface IgM, IgD, CD1c, CD24, CD19, CD20, CD21 and nuclear TFs, including Pax5, EBF, E2A and Oct2 (Melchers, 2015). Follicular B cells may circulate in the blood or reside in the primary and secondary follicles of spleen or lymph nodes. These cells take part in production of the bulk of high-affinity antibodies (Chaplin, 2010). They show expression of surface IgM, CD23, CD19, CD20, CD21 and CD22, and nuclear TF Pax5 (Melchers, 2015).

T-cell dependent activation of FO B cells leads to dynamic development of GCs within lymph nodes or spleen. Within GCs, the mature B cells undergo proliferation, differentiation, somatic hypermutation and class switching recombination, which results in generation of B lymphocytes with diverse specificity and antibody affinity (Chaplin, 2010). Antigen selected B cells that leave the GC may further become memory B cells or plasmablasts. GC B cells exhibit surface expression of CD20, CD38 and BR3, together with nuclear TFs BCL6, Pax5 and EBF (Melchers, 2015). Further differentiation of B cells generates short-lived plasmablasts and long-lived plasma cells (Chaplin, 2010). Differentiation of plasmablasts occurs early during infection and may be triggered either by a T cell-dependent or a T cell-independent activation leading to generation of plasma cells (Nutt *et al.*, 2015). Plasma cells are involved in later stages of infection and produce high affinity antibodies against antigens (Nutt *et al.*, 2015). Plasma cells are characterized by surface expression of CD45, CD138, TACI and/or BCMA, CD126, CD184 and CD320. They also express nuclear TFs, like BLIMP1, IRF4 and XBP1 (Melchers, 2015). When the same antigen is introduced next time, the presence of memory cells enables initiation of the secondary immune response which is rapid and more effective (Chaplin, 2010). During the GC response, memory B cells are selected later than those undergoing differentiation to plasma cells. As a result, memory B cells accumulate more immunoglobulin mutations and achieve higher affinity towards a specific antigen. After each round of antigen encounter the pool of differentially responding B cell clones expands and develops a stronger polyclonal response (Victoria *et al.*, 2012). Surface markers of memory B cells are CD20, CD27, CD80, CD84, CD86 and CD148, whereas nuclear TFs include OBF1 and SPI-B (Melchers, 2015).

Regulatory B (Breg) cell is another subtype of B lymphocytes. Bregs suppress function of the pro-inflammatory lymphocytes through the secretion of IL-10, IL-35, and TGF-β (Floudas *et al.*, 2016). Bregs also interact directly with T cells to promote their differentiation towards Tregs. Bregs can arise from most of the B cell types as a result of inflammatory signals

and BCR recognition mechanisms. Production of IL-10 is considered an important feature of Bregs. Large fraction of IL-10 producing B (B10) cells is found in the $CD19^+$ CD24 high $CD27^-$ CD38 high CD1d high CD5 transitional B cell population. Additionally, B10 cells express markers of activation and memory, CD48 and CD148, respectively (Floudas *et al.*, 2016). Purinergic molecules CD73 and CD39 have been also identified as markers of Bregs. Expression of these molecules allows Bregs to breakdown ATP to Ado and eventually suppress proliferation of T cells (Saze *et al.*, 2013).

In the past two decades much attention has been paid to the role of purinergic signaling in the immune system (Dubyak, 2009; Sakowicz-Burkiewicz & Pawelczyk, 2001; Dubyak, 2012; Rayah *et al.,* 2012; Jacob *et al.,* 2013; Burnstock & Boeynaems, 2014; Di Virgilio & Vuerich, 2015).

## PURINERGIC SIGNALING IN THE IMMUNE CELLS

Immune cells of different type and lineage express both types of purinergic receptors (Di Virgilio & Vuerich, 2015). The P1 receptors are widely expressed by immune cells of the myeloid and lymphoid origin, and vast numbers of data confirm the important role of $A_{2A}$-AR and $A_{2B}$-AR receptors in the control of inflammation (Di Virgilio & Vuerich, 2015). $A_{2A}$-AR and $A_{2B}$-AR receptors trigger production of immunosuppressive cAMP (Sakowicz-Burkiewicz *et al.*, 2011; Di Virgilio & Vuerich, 2015). P2X receptor expression has been observed in mononuclear phagocytes, neutrophils, eosinophils, T and B lymphocytes, NK cells and mast cells (Di Virgilio & Vuerich, 2015). P2YRs are widely expressed in the immune cells and have been thoroughly examined in neutrophilic and eosinophilic granulocytes, monocytes, macrophages, dendritic cells, T and B lymphocytes and NK cells (Di Virgilio & Vuerich, 2015). Purinergic receptors are involved in the chemotaxis of inflammatory and dendritic cells, migration of monocytes/macrophages, killing of bacteria by macrophages, secretion of IL-1β, maturation of dendritc cells and their enhanced antigen endocytosis, TCR-mediated activation of T lymphocytes, and suppression of the T cell activity (Burnstock & Boeynaems, 2014; Cekic & Linden, 2016). Relatively less is known about the role of the purinergic signaling in B cells. The main goal of this review is to summarize current knowledge on the impact of purinergic signaling on the B cell function.

## EXPRESSION OF P1 AND P2 RECEPTORS IN B CELLS

Comparative analysis of $A_{2A}$-AR expression between populations of lymphocytes revealed that this type of receptor is more abundant on T cells when compared to B cells (Koshiba *et al.*, 1999). It has been also reported that B cells exhibit surface expression of the $A_3$-AR receptor, which becomes overexpressed after activation of B cells (Gessi *et al.*, 2004). In B cells originating from humans and rats, expression of all four types of AR has been detected (Sakowicz *et al.*, 2009; Sakowicz-Burkiewicz *et al.*, 2012). Sluyter and coworkers have shown the presence of $P2X_1$, $P2X_2$, $P2X_4$ and $P2X_7$ on surface of B cells (Sluyter *et al.*, 2001), while Lee and colleagues have detected mRNA for each P2 receptor subtype (Lee *et al.*, 2006). The transcript levels of each P2X and P2Y were similar, except for $P2X_3$ and $P2X_7$, which were significantly lower (Lee *et al.*, 2006). In another study, mRNA of each P2Y subtype was observed, however, $P2Y_2$ and $P2Y_{12}$ were expressed the most abundantly, while $P2Y_4$ the most weakly (Wang *et al.*, 2004). Examination of $P2X_1$, $P2X_4$ and $P2X_7$ transcripts revealed the dominant expression of $P2X_4$ which was opposite to observations of Lee (Wang *et al.*, 2004; Lee *et al.*, 2006). Blood cells transformed with the Epstein Barr virus (EBV) into lymphoblastic cell lines are considered to be a valuable tool in examination of the immune system. It has been shown that EBV mediated transformation of B cells led to suppression of most of the P2 subtypes and upregulation of $P2X_7$ expression (Lee *et al.*, 2006). Such plasticity in the P2 expression should be considered when choosing an appropriate model for research on purinergic signaling in immune cells.

## DISTRIBUTION OF Ado AND ATP IN B CELL ENVIRONMENT

Concentration of Ado and ATP in a local environment of B cells is regulated by a network of membrane ectoenzymes (nucleotidases, deaminases, kinases) and transport proteins (Yegutkin *et al.*, 2002; Sakowicz-Burkiewicz *et al.*, 2010). Two antagonistic ectoenzymatic pathways have been observed at the surface of B cells, one relies on constitutive secretion of ATP and continuous resynthesis of high energy phosphates, while the second one depends on degradation of nucleotides to nucleosides and their subsequent inactivation (Yegutkin *et al.*, 2002; Sakowicz-Burkiewicz *et al.*, 2013a).

B cells continuously release ATP, which is subsequently degraded by surface ectoenzymes to Ado (Yegutkin *et al.*, 2002; Sakowicz-Burkiewicz *et al.*, 2010; Sakowicz-Burkiewicz *et al.*, 2013a). Experiments conducted on human naive and memory B cells, as well as on lymphoblastic cell lines, revealed that stimulated B cells release ATP by late endosomal/lisosomal vesicles through $Ca^{2+}$- and TI-VAMP protein-dependent mechanism (Schena *et al.*, 2013). Ectonucleoside triphosphate diphosphohydrolase-1 (NTPDase1, CD39) is an ectonucleotidase which hydrolyses nucleoside triphosphates (NTPs) on the surface of B cells and transforms ATP into adenosine diphosphate/monophosphate (ADP/AMP). Subsequently, ecto-5'-nucleotidase (5'-NT, CD73) hydrolyses AMP to Ado (Yegutkin *et al.*, 2002; Sakowicz-Burkiewicz *et al.*, 2010; Sakowicz-Burkiewicz *et al.*, 2013a). Concentration of Ado in the pericellular space of B cells is maintained at a constant level because of uptake through specific transporters and subsequent deamination to inosine (Ino) catalyzed by membrane and intracellular adenosine deaminase (ADA, CD26) (Herrera *et al.*, 2001; Sakowicz-Burkiewicz *et al.*, 2010; Sakowicz-Burkiewicz *et al.*, 2013a). Therefore, pericellular concentration and bioavailability of Ado for P1 receptors is determined by equilibrium between the rates of its synthesis and elimination (Herrera *et al.*, 2001; Sakowicz-Burkiewicz *et al.*, 2010).

Parallel to ATP consuming pathway, an opposite axis exists which promotes the increase in pericellular ATP concentration (Yegutkin *et al.*, 2002; Sakowicz-Burkiewicz *et al.*, 2010; Sakowicz-Burkiewicz *et al.*, 2013a). On the cell surface, besides being hydrolyzed to Ado, AMP may also be transphosphorylated to ADP by an ecto-

adenylate kinase (AK1β) (Yegutkin *et al.*, 2002; Sakowicz-Burkiewicz *et al.*, 2013a). ATP provides the main source of phosphates for both enzymes and may derive from the cell's surroundings or a degradation resistant pool in the membrane domains of B cells (Yegutkin *et al.*, 2002). ATP, resistant to enzymatic degradation by apyrase, is transported from cytosol to the membrane microdomains in lipid rafts probably through plasmatic or exocytotic vesicles (Yegutkin *et al.*, 2006). Eventually, ADP is converted into ATP in a reaction catalyzed by ecto-NDP-kinase (NDP) and this enzyme exhibits a much higher activity when compared to AK1β (Yegutkin *et al.*, 2002; Sakowicz-Burkiewicz *et al.*, 2013a). Aside from ATP, other nucleotide triphosphates have been also shown to provide phosphates for ecto-NDP (Yegutkin *et al.*, 2002; Sakowicz-Burkiewicz *et al.*, 2013a). Antagonistic ectoenzymatic pathways at the B cell membrane are regulated by concentration ratios of nucleoside mono-, di- and triphosphates and by the activities of the enzymes involved. Both factors determine the shift of reaction equilibrium towards degradation of nucleotides or towards their synthesis, resulting in activation of P1 or P2 receptors, respectively.

Processing of purinergic signaling messengers may not be the only function of B cell surface ectoenzymes, as it is possible that they modulate the action of purinergic receptors. The study on function of ADA in T cells conducted by Herrera and co-workers supported such an assumption (Herrera *et al.*, 2001). It has been shown that the membrane ADA is coupled to $A_{2B}$ receptor in T lymphocytes and their interaction enhanced the $A_{2B}$-AR affinity for its ligand, namely 5'-*N*-ethylocarboxyamideadenosine, and subsequent production of c-AMP. Moreover, inhibition of the ADA activity did not impair the synergistic effect of ADA-$A_{2B}$-AR coupling (Herrera *et al.*, 2001). However, similar observations in B cells have not been confirmed yet. Concentration of B cell pericellular Ado may also be controlled in a nonenzymatic manner by the transport of Ado through plasma membrane. Two types of membrane spanning nucleoside transporting channels take part in this process, i.e. equilibrative nucleoside transporters (ENT) and sodium cation dependent concentrative nucleoside transporters (CNT) (Cass *et al.*, 2002; Podgorska *et. al.*, 2005; Molina-Acras *et al.*, 2009). It has been shown that peripheral blood mononuclear cells exhibit expression of CNT2, CNT3, ENT1 and ENT2, however, the Ado uptake is mediated predominantly by ENT1 (Molina-Acras *et al.*, 2003). The expression level of particular nucleoside transporters varies in B cells and depends on their physiological state. Experiments performed on rat B cells indicated that impaired nucleoside transport that takes place in diabetic B lymphocytes results from alterations in the expression of rENT1, rENT2 and rCNT2 transporters, which are independently and differentially regulated by glucose and insulin (Sakowicz *et al.*, 2005). Nonetheless, data focusing particularly on the nucleoside transport in B cells are still limited.

## EFFECTOR ROLE OF Ado AND ATP IN B CELL FUNCTION

### B cells regulate T cell function through purinergic signaling

Recent data suggests that Bregs utilize purinergic signaling to control the function of T cells. Adenine nucleotides and nucleosides activate different signaling pathways, and thus appropriate ratio of Ado and ATP concentrations needs to be maintained in the environment of B cells. Study on impact of ATP and Ado on B and T cells cultured separately and in a co-culture suggested that B cells regulate their own function and function of T cells through purinergic signaling (Saze *et al.*, 2013). Ado derived from enzymatic degradation of ATP suppresses B cell activity in an autocrine fashion through $A_3$-AR, which results in lack of a TCR-dependent response of $CD4^+$, $CD8^+$ T-cells (Saze *et al.*, 2013). On the contrary, the activated B cells become $CD39^{high}$ $CD73^{low}$ and increase production of AMP (Saze *et al.*, 2013). AMP produced by B cells enhances their proliferation in an autocrine manner and at the same time it inhibits proliferation of T cells in a paracrine manner (Saze *et al.*, 2013). Moreover, activated B cells exhibit an increased expression of ADA, which results in further elimination of Ado (Saze *et al.*, 2013). A study on purinergic signaling in Bregs showed that suppression of $CD4^+$ T cell proliferation was exerted under *in vivo* condition only by subset of $CD39^{high}$ $CD73^+$ $CD24^+$ $CD25^{high}$ and $CD38^{high}$ B cells (Figueiro *et al.*, 2016). This specific fraction of B cells was also capable of producing GM-CSF, TNF-α, IL-6 and moderate levels of IL-10. Such cell phenotype allows vigorous production of 5-AMP and ADO, and subsequent suppression of the T effector cells (Teff). Breg-derived ADO interacts with $A_{2A}$-AR on Teffs, leading to cAMP accumulation and depletion of their effector function. Moreover, autocrine ADO, AMP, GM-CSF, TNF-α and IL-6 promote expansion of the CD39 high B cells. ADO and AMP promotes proliferation of Bregs through $A_{1A}$-AR and $A_{2A}$-AR, whereas GM-CSF and IL-6 have been reported to promote differentiation of B cells to Bregs (Deng *et al.*, 2012; Rosser *et al.*, 2014; Figueiro *et al.*, 2016). Increase in proportion of CD39 high B cells leads to elevation of AMP and ADO levels, and subsequent suppression of activation and proliferation of $CD4^+$ Teff cells (Figueiro *et al.*, 2016). It was proposed that purinergic regulation of Bregs is independent of IL-10 production (Wang *et al.*, 2014). However, recent study demonstrated that IL-$10^{(-/-)}$ B cells showed decreased expression of CD73 and resulted in an impaired Ado production (Kaku *et al.*, 2014).

### B cells achieve immunocompetence through purinergic signaling

Resent study demonstrated that Ado synthesis is necessary for optimal development, implantation and maintenance of the plasmocyte population in bone marrow in the course of primary immune response in *Mus musculus* (Conter *et al.*, 2014). B cells differentiate into plasmocytes in the GCs and during primary immune response the maturing germinal centers become highly enriched in B cells $CD73^+$ and follicular helper T cells ($T_{FH}$) $CD73^+$ (Conter *et al.*, 2014). $T_{FH}$ cells play a key role in generation and maintenance of germinal centers and in differentiation of plasma cells and memory B cells (Crotty, 2011; Conter *et al.*, 2014). Moreover, it has been shown that activity of CD73 in GCs leads to proper generation of the bone marrow plasmocyte population (Conter *et al.*, 2014). On the other hand, suppressed CD73 expression in B and $T_{FH}$ cells maybe efficiently compensated by other populations of cells (Conter *et al.*, 2014). Researchers hypothesized that arise in CD73 expression in GCs

may be the response to hypoxia and an increased level of ATP that naturally occurs in GCs during intense processes of proliferation and apoptosis (Conter *et al.*, 2014). Ado also plays an important role in immunoglobulin class switching (ICS), which is a key mechanism of humoral immune response resulting in differentiation of immunoglobulin isotypes (Schena *et al.*, 2013). It has been observed that stimulation of BCR and toll-like receptor (TLR) in $CD39^+/CD73^+$ and $CD39^+/CD73^-$ subpopulations of human naive and memory B cells resulted in the release of ATP and subsequent Ado formation (Schena *et al.*, 2013). CD73 expression promotes higher frequency of ICS to IgG or IgA in both, the naive and memory B cells (Schena *et al.*, 2013). Additionally, immunophenotype $CD73^+$ is associated with a higher surface expression of CD180, a TLR receptor homolog which regulates B cell sensitivity to TLR9- and CD40L-dependent stimulation (Schena *et al.*, 2013). Moreover, comparing to $CD73^-$ B cells, memory $CD73^+$ cells exhibited an increased expression level of Xbp-1, a transcription factor taking part in later stages of B cell development (Schena *et al.*, 2013). Naive and memory $CD73^+$ B cells cultured with either ADA or CD73 inhibitor, upon stimulation showed significant disruption of class switch recombination when compared to untreated cells (Schena *et al.*, 2013). On the other hand, naive and memory $CD73^-$ B cells cultivated with Ado, upon stimulation exhibited a significantly higher number of class switched IgG/IgA B cells when compared to $CD73^-$ lymphocytes cultured in a medium deprived of Ado (Schena *et al.*, 2013). Release of immunoglobulins, however, may depend on ATP signals. $P2X_7$ activation has been shown to be involved in a T cell-independent production of IgM by B lymphocytes (Sakowicz-Burkiewicz *et al.*, 2013b). Stimulation of B cells with *Staphylococcus aureus* Cowan strain I (*SAC)* and IL-2 promotes IgM production and administration of BzATP, a $P2X_7$ agonist, significantly enhanced immunoglobulin secretion (Sakowicz-Burkiewicz *et al.*, 2013b). On the other hand, selective inhibition of $P2X_7$ in stimulated B cells resulted in a total inhibition of IgM release (Sakowicz-Burkiewicz *et al.*, 2013b). However, further investigation needs to be conducted to reveal a specific role of purinergic signaling in the T cell-independent response of B cells.

### Ado and ATP control the rolling of lymphocytes during inflammation

Traffic of lymphocytes between blood and tissues is essential for propagation of inflammation and purinergic signaling plays an important role in this process through conditioning kinetics of lymphocytes (Henttinen *et al.*, 2003; Yegutkin *et al.*, 2014). Extravasation of leukocytes to target tissue consists of following steps: temporal adhesion of lymphocytes to vascular endothelium, rolling of cells along endothelium, activation resulting in holding of the cells and subsequent transmigration into tissue (Springer, 1994). Leukocyte-endothelial interactions are regulated via cellular adhesion and purinergic signalization (Panes *et al.*, 1999). Comparison of different endothelial and lymphoid cell lines has shown that endothelial cells exhibit high activity of CD73 and CD39, which results in a phenotype promoting Ado synthesis, whereas action of lymphoid cells is directed towards pericellular ATP accumulation and Ado elimination (Yegutkin *et al.*, 2002; Henttinen *et al.*, 2003; Yegutkin *et al.*, 2006). B lymphocytes exhibit low or lack of CD39 and CD73 activity, moreover, it has been shown that B cells from the Namalwa leukemic cell line, as well as B lymphocytes isolated from blood of healthy donors, inhibited the CD73 activity of the HUVEC endothelial cell line (Yegutkin *et al.*, 2002; Henttinen *et al.*, 2003; Yegutkin *et al.*, 2006). However, the mechanism governing the inhibition of CD73 activity has not been identified yet (Henttinen *et al.*, 2003). It has been observed that inhibition of endothelial CD73 by B cells was associated with elimination of extracellular Ado owing to ecto-ADA activity (Henttinen *et al.*, 2003). Moreover, it has been shown that the Namalwa lymphocytes used apyrase resistant pericellular pool of ATP as source of γ-phosphates in continuous conversion of AMP to ADP, thus limiting availability of AMP for endothelial CD73 (Henttinen *et al.*, 2003). It can be concluded that a proper course of events constituting inflammatory response, such as leukocyte-endothelial adhesion and subsequent migration of leukocytes to tissues, is determined by B cell dependent shift of local metabolism towards elimination of Ado and accumulation of ATP (Henttinen *et al.*, 2003). Removal of Ado and prevention of its synthesis is controlled at three levels: inhibition of endothelial CD73, catalytic deamination of residual Ado and continuous elimination of substrate for CD73 *via* conversion of pericellular AMP to ADP (Henttinen *et al.*, 2003). It is assumed that such phenotype allows B cells to avoid suppressive impact of Ado and to sustain micromolar halo of ATP which leads to propagation of ATP signaling in endothelial cells during inflammation (Yegutkin *et al.*, 2002; Henttinen *et al.*, 2003; Yegutkin *et al.*, 2006).

Accumulation of pericellular ATP occurring in B cells activates the $P2X_7$ receptor, resulting in shedding of CD21, CD23 and CD62L from the cell surface (Frémeaux-Bacchi *et al.*, 1998; Gu *et al.*, 1998; Venturi *et al.*, 2003; Sengstake *et al.*, 2006). Disappearance of these molecules modulates immune responses at different levels. Adhesion protein L-selectin (CD62L) is expressed on the surface of B and T cells (Gu *et al.*, 1998). Interaction of CD62L with epitopes on the endothelial venules is the first event in the adhesion cascade leading to integrin interactions and transendothelial migration of leukocytes (Gu *et al.*, 1998). It has been shown that $P2X_7$ stimulation activates the membrane matrix metalloproteinases (MMPs), resulting in the cleavage of CD62L at a proximal extracellular domain and subsequent shedding of soluble CD62L (Gu *et al.*, 1998). Deletion of MMPs cleaved sequence on the proximal membrane domain of CD62L results in continuous migration of activated lymphocytes to peripheral lymph nodes, instead of temporal rolling and movement into thymus, which normally occurs after CD62L shedding (Venturi *et al.*, 2003). CD23 is a membrane spanning protein of type II, acting as a low affinity receptor for IgE (FcεRII), moreover, it also functions as adhesion molecule involved in transendothelial movement of B cells (Sengstake *et al.*, 2006). CD23 becomes overexpressed in activated B cells and sheds after $P2X_7$ activation, however, it sheds much slower than CD62L (Sengstake *et al.*, 2006). It has been also shown that the level of CD23 decreases in B cells during migration along the layer of vascular endothelium (Gu *et al.*, 1998; Sengstake *et al.*, 2006). CD21 is a part of membrane coreceptor complex CD19/CD81/Leu-13. The coreceptor complex interacts with BCR to lower the B cell activation threshold (Cherukuri *et al.*, 2001). Activation of $P2X_7$ by

**Table 1. Table contains alternations in B cell purinergic signaling which are related to certain disorders: diabetes and common variable immunodeficiency.**
Experimental model column informs if cell lines or primary cultures were examined. Appropriate references are listed in the last column

| Disease | Alternation in B cell purinergic signaling | Experimental model | References |
|---|---|---|---|
| Diabetes | Drop in membrane ADA activity | SKW6.4 cell line cultured at high glucose concentrations | Kocbuch *et al*., 2009 |
| | Drop in expression of A1-, A2B- and A3AR | Rat B cells cultured at high glucose concentrations | Sakowicz-Burkiewicz *et al*., 2009 |
| | Increase in expression of A1- and A2AR; Drop in expression of A2BAR | Rat B cells cultured at elevated insulin concentrations | Sakowicz-Burkiewicz *et al*., 2010 |
| | Impaired ATP-P2X7 dependant IgM release; Drop in production and release of intracellular ATP | Isolated human B cells cultured at high glucose concentrations | Sakowicz-Burkiewicz *et al*., 2013b |
| Common variable immunodeficiency (CVID) | Drop in surface expression of CD73 | Naive, IgM memory and class switched B cells isolated from CVID patients | Schena *et al*., 2013 |

ATP results in MMPs mediated proteolytic cleavage of CD21 and its shedding from periphery of B cells (Sengstake *et al*., 2006). Soluble form of CD21 interacts with complement fragments and forms complexes with CD23 trimers, thereby inhibiting CD23 dependent synthesis of IgE (Frémeaux-Bacchi *et al*., 1998). ATP is thus a modulator of B cell migration along endothelium, activation and IgE synthesis.

## SUMMARY AND PERSPECTIVES

The expression profile of purinergic signaling components, such as purinoreceptors, specific ectoenzymes and nucleoside transporters has been examined extensively in B cells. Function of many of those components has been also revealed. The B lymphocytes remain inactivated under high pericellular concentration of Ado. After activation, B cells increase production of AMP to enhance their own proliferation. Metabolism of activated B cells is shifted towards elimination of Ado and accumulation of pericellular ATP, which enhance the adhesion of lymphocytes to vascular endothelium and subsequent migration to target tissues. $P2X_7$-dependent shedding of surface molecules CD21 and CD62L has been shown to be involved in transendothelial migration of B cells. There is also evidence showing that $P2X_7$ is directly involved in the release of IgM from B cells after TI activation. Although ATP signaling is dominant in activated B lymphocytes, Ado seems to be crucial in achieving immunocompetence by activated cells. It has been observed that maturation of plasmocytes in germinal centers, as well as successful CSR, depends on elevated expression of CD73 in the B cell environment. Disrupted expression of P1 receptors and enzymes metabolizing Ado and ATP has been observed in a course of CVID and diabetes (Table 1). Action of $A_1$-AR, $A_{2A}$-AR, $A_{2B}$-AR, $A_3$-AR and $P2X_7$ receptors in B cells has been so far investigated the most extensively, however, insight into function of other P2 receptors is necessary.

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