Cell. Mol. Life Sci. 65 (2008) 2528 – 2540
1420-682X/08/162528-13
DOI 10.1007/s00018-008-8095-1

Cellular and Molecular Life Sciences

Review

Purinergic regulation of neutrophil chemotaxis

W. G. Junger

Department of Surgery, Beth Israel Deaconess Medical Center and Harvard Medical School, 330 Brookline Avenue, East Stoneman 8M 10C, Boston, Massachusetts 02215 (USA), e-mail: wjunger@bidmc.harvard.edu

Received 25 February 2008, received after revision 31 March 2008, accepted 02 April 2008
Online First 9 May 2008

Abstract. Chemotaxis allows polymorphonuclear neutrophils (PMN) to rapidly reach infected and inflamed sites. However, excessive influx of PMN damages host tissues. Better knowledge of the mechanisms that control PMN chemotaxis may lead to improved treatments of inflammatory diseases. Recent findings suggest that ATP and adenosine are involved in PMN chemotaxis. Therefore, these purinergic signaling processes may be suitable targets for novel therapeutic approaches to ameliorate host tissue damage.

Keywords. Neutrophils, inflammation, chemotaxis, puringeric receptors, tissue damage.

Introduction

Up to 70 % of all leukocytes in the peripheral blood of healthy adults are polymorphonuclear neutrophils (PMN). PMN play an important role in the innate immune defense. They are well equipped to detect the presence of invading micro-organisms, to accurately locate such invaders at sites of infection, and to rapidly migrate to these sites where PMN kill the invaders by unleashing a powerful set of defensive tools that include reactive oxygen species (ROS), proteolytic enzymes, and bactericidal mediators stored in intracellular granules of PMN or deposited into the extracellular space in DNA-containing structures that have been termed NETs [1 – 3]. PMN liberate these cytotoxic agents to incapacitate and destroy micro-organisms, but often host tissues are also compromised in the process. In fact, collateral damage to host tissues by excessively activated PMN is a hallmark of chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, and asthma [4 – 6]. During acute inflammatory episodes caused by shock, ischemia, and reperfusion, PMN-induced tissue damage is responsible for severe clinical complications such as acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) - leading causes of death in trauma patients suffering post-traumatic shock [7 – 10].

It is unclear what triggers this undiscriminating and overzealous response of PMN. Therapeutic approaches to treat these inflammatory diseases may target a wide range of processes that are involved in the initial activation of PMN or their recruitment to inflamed tissues; other approaches focus on the neutralization of cytotoxic mediators like elastase that are released from activated PMN. Among these potential therapeutic targets, PMN recruitment to inflamed tissues is the most promising. Indeed, a number of drugs have been developed to prevent the sequestration of PMN into affected host tissues [11 – 15]. Of particular interest is PMN chemotaxis, as this is a key process involved in the recruitment of PMN to sites of inflammation. However, a more complete understanding of the mechanisms that control PMN chemotaxis is required to develop novel therapeutic strategies that effectively reduce chemotaxis and PMN-induced tissue damage.

Life cycle of PMN

PMN are formed by continuous differentiation of hematopoietic stem cells in the bone marrow through a process referred to as granulopoiesis. Myeloblasts differentiate into promyloblasts, myelocytes, and then metamyelocytes as well as segmented band neutrophils that can be found in circulation during stress [16–19]. Metamyelocytes are the precursors of polymorphonuclear leukocytes, which are commonly referred to as granulocytes, including eosinophils, basophils, and neutrophils (PMN). The newly generated PMN are stored in the bone marrow, from where they can be rapidly released into the blood stream when needed. This process is tightly regulated, allowing significant increases in granulopoiesis and in the circulating PMN population to facilitate host defense, when PMN turnover can increase ten-fold and PMN counts in peripheral blood can surge to levels several fold higher than under normal conditions. Granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (G-CSF), interleukin (IL)-3, IL-23, and IL-17 are key mediators that regulate the production of PMN [20, 21]. In addition to these regulatory mediators, the mechanisms that control the migration of PMN within the bone marrow as well as their delivery to the bloodstream determine the final rate at which PMN are dispatched in response to inflammation or infection.

Extravasation

Upon their release from the bone marrow, PMN can circulate in the vasculature for several hours before they are finally recruited to inflamed tissues in order to pursue microbial invaders or to eliminate dead or dying cells. Numerous different chemokines and chemoattractant mediators that are produced by bacteria or by infected and inflamed tissues control the recruitment of PMN from the bloodstream into affected tissues. Adhesion molecules such as E-, L-, and P-selectins facilitate ever tighter interactions of circulating PMN with the endothelial cell layer lining the blood vessels closest to inflamed or infected host tissues [2, 11]. Initially, P-selectin is rapidly mobilized to the cell surface of the endothelial cell layer coating blood vessels of the microcirculation. Adhesion molecules on PMN such as P-selectin ligand-1 (PSGL-1) are concentrated on the tips of microvilli that protrude from circulating PMN, allowing the PMN to interact with P-selectin, which results in tethering of PMN to the endothelium. Together with the vasodilatation of capillaries in inflamed tissues, this process of tethering slows circulating PMN, resulting in the "rolling" of PMN along the endothelium of the microcirculation. In this microenvironment, PMN can become stimulated by mediators such as platelet-activating factor (PAF), leukotriene B_4 (LTB_4), and IL-8 that are generated by the inflamed endothelium [11, 22]. Upon binding of these mediators to receptors on the cell surface of PMN, the cells express β_2-integrins (CD11/CD18 and LFA-1) stored in intracellular granules as well as a number of additional integrins such as $\alpha_4\beta_1$-integrins [23–25]. With the help of these integrins, PMN bind more tightly to the endothelium by recognition of endothelial adhesion molecules such as intercellular adhesion molecules (ICAMs) and vascular cell adhesion molecule-1 (VCAM-1) [23–25]. These PMN-endothelial interactions culminate in the firm adherence and spreading of PMN on the endothelium, which allows PMN to penetrate the endothelial cell layer in order to transmigrate into the extravascular space. Extravasation of PMN into the extravascular space is known to occur in a paracellular manner at endothelial cell junctions where molecules such as platelet endothelial adhesion molecule-1 (PECAM-1), expressed at these regions of endothelial cell layers, appear to play important roles in facilitating transmigration [26]. In addition to this paracellular transmigration process, transcellular migration of PMN across the endothelial cell layer can also occur. In the latter case, PMN seem to penetrate and travel through individual endothelial cells at non-junctional locations without impairing endothelial cell integrity [24, 25]. Once in the extravascular space, PMN migrate upstream in chemotactic gradient fields generated by chemotactic mediators that are released at inflamed sites (Fig. 1).

PMN chemotaxis

In contrast to random cell migration (chemokinesis), chemotaxis denotes the ability of cells to migrate in a directed fashion upstream in a chemotactic gradient field. This process requires coordinated and directional cytoskeletal rearrangements, mediated via F-actin polymerization, that produce pseudopods at the leading edge in the direction of the chemotactic gradient field [27]. In order to produce appropriate pseudopods that result in chemotaxis rather than merely in random membrane ruffling or in cell spreading, F-actin polymerization is restricted to the leading edge, while extension of the leading edge is synchronized with myosin responses that induce the retraction of cell membrane at the receding edge (uropod). This is accomplished through contractile forces generated by myosin motor proteins and the consequent interactions with actin filaments at

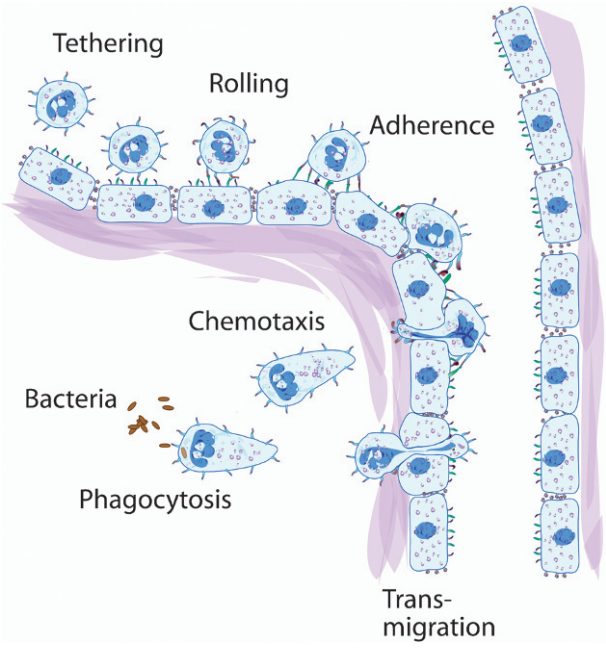

Figure 1. Recruitment of neutrophils to sites of infection or inflammation. Circulating neutrophils are slowed down close to sites of infection or inflammation by adhesion molecules expressed on the adjacent endothelium. This results in tethering and rolling of PMN along the blood vessel wall. Increasingly firm interactions of endothelial cells and PMN result in adhesion, followed by either pericellular or transcellular transmigration of PMN across the endothelial cell layer. Subsequently, PMN reach sites of inflammation or infection by chemotaxis along chemotactic gradient fields that guide them, for example to invading bacteria, which are phagocytosed and killed by oxygen radicals and lytic enzymes.

lateral and posterior regions of the cell cortex [27–29].

Chemotaxis requires at least three distinct processes that accomplish gradient sensing, polarization within the gradient field, and migration itself (locomotion). Gradient sensing is necessary to detect the orientation of the chemotactic gradient field. PMN accomplish this task by recognizing chemoattractant concentration differences in the extracellular space that surrounds the cells. PMN are capable of detecting minute concentration differences, and this makes it possible for PMN to recognize and orient in chemotactic gradients that can be as shallow as 1% across the length of the cell body. PMN are highly specialized to detect and distinguish numerous different chemoattractant substances that are produced by bacteria or inflamed host cells. To do so, PMN possess a variety of chemoattractant receptors. Among the best studied receptors are formyl peptide receptors (FPR), which allow PMN to recognize formylated peptides such as *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) that are released from bacterial cell walls. Other groups of chemoattractant receptors control PMN migration in response to inflammatory mediators such as IL-8, platelet activating factor (PAF), LTB_4, and complement fragment 5a (C5a). Once gradient sensing is accomplished, PMN polarize within the chemotactic gradient field. The cells assume an elongated morphology along the anterior-posterior axis in alignment within the chemotactic gradient field. Cell polarization enhances the efficiency and speed of the forward movement of cells [30].

Local excitation and global inhibition model of chemotaxis. Gradient sensing requires positive feedback loops at the leading edge, allowing for directional gradient sensing of PMN in a gradient field [31]. This involves "front"-specific responses at the region experiencing the highest concentrations of chemoattractants and "rear"-specific responses where chemotactic stimulation is lowest [32]. Mathematical models and experimental evidence indicate that cells must amplify weak signal differentials of chemoattractant gradients into sharp internal differences, most clearly demonstrated by the redistribution of intracellular proteins and lipids at the front (e.g., PI3K, PIP3, Rac, F-actin) or back (e.g., PTEN, myosin, Rho) of cells [30]. However, the mechanisms by which signal amplification occurs have been the subject of much debate and controversy [33, 34].

Most current models of chemotaxis place signaling via phosphoinositide-3 kinase (PI3K) and phosphatidylinositol (3,4,5)-trisphosphate (PIP3) at center stage. The distribution of PIP3 in the cytosol of PMN is 3–6 times steeper than the external chemoattractant gradient, indicating the existence of a powerful amplification mechanism that aids gradient sensing and allows PMN to respond to shallow chemotactic gradients [33]. Several mechanisms have been proposed to explain how such signal amplification might occur [35–37]. Some models suggest two distinct feedback loops: a stimulatory, positive loop that acts over a short range at the leading edge, and an inhibitory, negative feedback loop that acts over a longer range at the rear of the cell [34]. A large body of experimental evidence supports the existence of such positive and negative feedback loops that form the basis for the "*Local Excitation and Global Inhibition*" (LEGI) model, the most widely accepted chemotaxis model. The LEGI model incorporates the concept of opposing positive and negative regulatory feedback mechanisms and it can explain how cells remain responsive to further stimulation while they are in the process of migration [38]. The LEGI model suggests that a balance between excitation and inhibition controls membrane binding and activity of PI3K and PTEN. The dominant mechanisms that control PI3K and PTEN activity in migrating cells, however, are not well understood.

We found that ATP is released at the leading edge of PMN and that ATP and positive feedback through P2Y2 receptors are critical for proper gradient sensing and correct polarization of PMN. Released ATP is rapidly converted to adenosine, while A3 adenosine receptors are distributed to the leading edge, allowing a second stage of signal amplification that controls migration speed [39]. P2Y2 and A3 receptors can induce PIP3 and Rac activation, which is a characteristic feature associated with the leading edge of polarized cells. Thus, feedback via P2Y2 and A3 receptors may explain how signal amplification is accomplished in the LEGI model of chemotaxis. In order to adapt to changing chemoattractant concentrations as cells get closer to the chemotactic source, the chemotactic receptor system of PMN is gradually desensitized, allowing the cells to maintain their gradient sensing ability over a wide range of chemoattractant concentrations [33]. Desensitization is a hallmark of many purinergic receptors [40], providing further support for the notion that purinergic receptors are involved in the control of PMN chemotaxis.

ATP release. ATP has been long recognized as the molecular currency that provides the energy for virtually all cellular processes. Intracellular concentrations of ATP are very high, ranging from 3–10 mM. By contrast, extracellular ATP concentrations are considerably lower. Physiological ATP concentrations in plasma are lower than 1 μM under normal conditions. Higher extracellular ATP concentrations are found under pathophysiological conditions, such as hypoxia and ischemia, that lead to cell damage, loss of cell viability, and cytolysis that results in spillage of cellular contents, including ATP, into the extracellular surroundings [41, 42]. ATP is also released by bacteria, which may help guide PMN and other inflammatory cells to sites of infection.

In recent years it has become clear that, under normal physiological conditions, intact cells also can release ATP in a deliberate and controlled fashion [43–47]. Increasing evidence suggests that released cellular ATP and the phosphohydrolytic products generated from ATP regulate cell functions via autocrine and paracrine feedback mechanisms [48–52]. Yet, the mechanisms by which mammalian cells release ATP under these conditions are still unclear. There are two major theories as to how ATP release is accomplished. The best documented mechanism involves exocytosis of secretory granules that contain ATP. This process is observed in neurons, pancreatic cells, mast cells, and aggregating platelets [53–58]. The second most widely accepted, yet less well understood, mechanism involves ATP release through specialized channels. Such channel-mediated release mechanisms have been proposed to facilitate ATP discharge from red blood cells, epithelial cells, smooth muscle cells, and cardiac muscle cells in response to mechanical and osmotic stimulation. Direct mechanical perturbations of the cell membrane or membrane deformation resulting from osmotic cell swelling or cell shrinkage cause the opening of mechano-sensitive channels that permit ATP to be released into the extracellular environment [48, 49, 56, 59–62]. Work from this laboratory has shown that mechanical stimulation and osmotic stress, induced by the exposure of cells to hypertonic saline, cause rapid release of ATP from human lymphocytes and PMN [63, 64]. The released ATP and its hydrolytic product, adenosine, regulate the functional responses of these cells to osmotic stress via purinergic receptors that are expressed on the cell surface of PMN and lymphocytes [64–66]. These findings are of clinical interest and have encouraged the increasingly wide-spread use of hypertonic resuscitation fluids to modulate the inflammatory response in trauma patients [67–70].

During PMN chemotaxis, highly dynamic cell membrane deformation occurs predominantly at the leading edge of migrating cells. The mechanical stress exerted on the cell membrane at the leading edge is likely to cause ATP release through mechano-sensitive channels. This raises the questions whether ATP release at the leading edge may be involved in the control of chemotaxis. In support of this concept we found that chemotactic stimulation of PMN causes rapid ATP release at the leading edge and that extracellular ATP concentrations close to the cell surface appear to correlate with the extent of pseudopod activity at different membrane regions of stimulated PMN [39]. However, it is still unclear how ATP release occurs under these circumstances. The involvement of connexin hemichannels, specifically connexin 43, has been proposed [71]. It is possible that several other ATP release mechanisms also may be involved, as is suggested by preliminary results with a recently developed microscope method that allows us to visualize ATP release from living cells [72]. Maxi-anion channel activity has been identified in several cell types including cells of the immune system [52, 73–76]. This channel type has been shown to facilitate ATP release from the macula densa, allowing osmotic sensing in the kidney [77, 78]. The molecular nature of a maxi-anion channels was first discovered a few years ago, when *tweety*, a gene located in *Drosophila* 'flightless,' was found to resemble mammalian maxi-anion channels. Subsequently, three human maxi-anion channels (tweety homolog hTTYH1–3) were identified by Suzuki et al. [79, 80]. Two isoforms, hTTYH1 and hTTYH3, are activated by mechanical stress and these proteins are

expressed in a number of human tissues, including in peripheral blood leukocytes [79]. Thus, it is possible that hTTYH could be involved in ATP release from PMN.

Finally, degranulation, which plays such an integral role in PMN physiology, is another likely mechanism that may facilitate ATP release from PMN. Cytoplasmic granules have been observed to concentrate at the leading edge of migrating cells, where these granules appear to contact the cell membrane and release their contents [81, 82]. It is possible that these PMN granules may contain ATP, as is the case in other cell types. In platelets, for example, dense granules store ADP and ATP, which play central roles in amplification of platelet aggregation and modulation of the functions of vascular endothelial cells and leukocytes [83].

Purinergic receptors

Since the discovery by Geoffrey Burnstock and his coworkers that intact cells can release ATP, a large number of mammalian purinergic receptors have been identified and characterized [43]. Family members of these receptors are expressed in cell type-specific distribution patterns on virtually all mammalian cells, including PMN. Purinergic receptors, also termed P2 or nucleotide receptors, are divided into two major subfamilies: the GPCR-type P2Y receptors, of which eight unique isoforms have been cloned and characterized (P2Y1, 2, 4, 6, 11, 12, 13, and 14); and the P2X receptors, which are ligand-gated ion channels with seven members (P2X1–7). The P2Y receptor subtypes trigger different signaling events, depending on the G proteins with which each is associated [43]. PMN have been reported to express the P2Y1, 2, 4, 6, 11, and 14 and P2X1, 4, 5, and 7 receptor subtypes [84, 85]. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) analysis has indicated that P2Y2 seems to be the most abundantly expressed P2 receptor subtype in human PMN and HL60 cells, a PMN-like cell line that is often used to study chemotaxis [39]. P2Y2 receptors are G_i coupled and can activate PIP3 and Rac [86, 87], molecules that are tightly associated with "front"-specific cytoskeletal responses during cell polarization and chemotaxis [34].

The hydrolytic product of ATP, adenosine, is an important endogenous regulator of neutrophil functions that can modulate neutrophil activity by interacting with specific adenosine receptors on the cell surface of PMN. These adenosine receptors, also termed P1 receptors, are GPCRs and they are comprised of four unique subtypes (A1, A2a, A2b, and A3) [43]. All four P1 receptor subtypes have been reported in PMN [85]. However, recent real-time RT-PCR analysis suggests that human PMN and HL60 cells express predominantly A2a and A3 receptors [39]. These two adenosine receptor subtypes have opposing effects on the activation and functional responses of PMN. Both P1 receptor subtypes may thus cooperate to fine-tune responses of PMN to extracellular adenosine. A2a receptors are linked to G_s and the cyclic AMP/protein kinase A (cAMP/PKA) pathway, which is well known to suppress PMN responses. A3 receptors, on the other hand, stimulate PI3K pathways that up-regulate PMN responses and link to key signaling events that are associated with PMN chemotaxis [88, 89].

We observed that in resting cells, A3 receptors appear to be located primarily in intracellular compartments where they seem to associate with granules. Upon cell stimulation with chemoattractant, A3 receptors are rapidly mobilized to the cell surface of PMN [39]. Interestingly, cell polarization leads to the accumulation of A3 receptors at the leading edge, suggesting that A3 receptors are involved in regulating chemotaxis at the front of cells (Fig. 2). In contrast, A2a receptors are uniformly distributed across the cell surface and cell polarization does not seem to change this distribution pattern. This suggests that the inhibitory A2a receptors may function to globally suppress pseudopod formation across the entire cell surface of PMN, except at the leading edge, where A3 receptors counteract the suppressive action of A2a receptors. Thus, it is possible that A2a receptors may play a role in amplifying gradient signals by suppressing chemotactic receptor signaling at membrane regions furthest away from the leading edge. This possibility is supported by the large difference in ligand affinities of the A2a and A3 receptors. A2a receptors are several orders of magnitude more sensitive to adenosine than are A3 receptors [43]. Thus, differences in external adenosine concentrations in the environment that surrounds migrating PMN may further contribute to the regulation of chemotaxis by promoting responses via A3 receptors at the leading edge, while suppressing responses via A2a receptors at other cell membrane regions.

Ecto-nucleotidases and adenosine formation. The importance of adenosine in controlling PMN chemotaxis indicates that the mechanisms that convert ATP to adenosine are equally important in regulating PMN responses. Virtually all cell types, including PMN, express on their cell surface ecto-nucleotidases [90–92]. The large group of ecto-nucleotidases include the ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) family, the ecto-nucleotide pyrophospha-

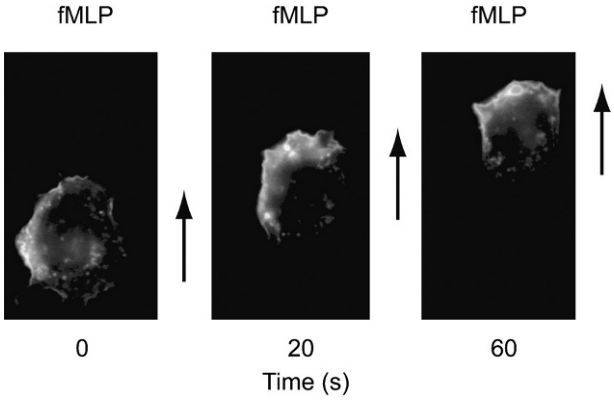

Figure 2. Fluorescence image of a migrating HL60 cell expressing EGFP-A3 receptor fusion protein. Cells were stably transfected to express EGFP-A3 adenosine receptor fusion protein, differentiated to acquire PMN-like characteristics, and subjected to a chemotactic gradient field that was generated by releasing fMLP from a micropipette (top).

tase/phosphodiesterase (E-NPP) family, the alkaline phosphatases (ALP), and ecto-5'-nucleotidase (CD73) [54, 93–98]. E-NTPDases are comprised of seven different isoforms and all are efficient enzymes with high turnover rates (NTPDase1–6, 8; also referred to as ENTPD, ATPDase, or CD39) that hydrolyze ATP or ADP to AMP. Enzymes of the E-NPP family, which can hydrolyze ATP to AMP and PiPi pyrophosphate, consist of three members (NPP1–3). A splice variant of NPP2, autotaxin, is soluble and converts AMP to adenosine. Alkaline phosphatases (ALP), which consist of four family members, and ecto-5'-nucleotidase (CD73) catalyze the conversion of nucleotides to adenosine [97].

PMN have been shown to express E-NTPDases and CD73 [85]. PMN effectively hydrolyze extracellular ATP to AMP and adenosine [39, 71], suggesting that PMN possess an efficient repertoire of ecto-nucleotidases. Although NTPDases likely play a role in this process [71, 99, 100], little is known about which ecto-nucleotidase members are expressed in PMN. Some time ago it was shown that ecto-enzymes are present in organelles and on the cell surface of PMN. Interestingly, the enzymatic activities of these proteins and their subcellular distributions were found to change with cell stimulation [101–104], suggesting that these enzymes play a role in cell activation.

Of the four ALP isoforms, PMN express the liver/bone/kidney-type (non-tissue specific) alkaline phosphatase [105]. Interestingly, ALP activity in PMN of patients with various diseases is significantly different from that of healthy controls [106]. In addition, a potential involvement of ALP in the regulation of PMN chemotaxis *in vivo* is supported by clinical evidence, which shows that diminished ALP activity may go hand-in-hand with a loss of PMN chemotaxis and an increased incidence of infectious complications [107–109]. ALP is present in the membranes of secretory vesicles that readily translocate from the cytoplasm to the cell membrane [108]. Thus, the activation of PMN may increase the presence of ALP on the cell surface, potentially allowing ALP to modulate PMN chemotaxis by generating extracellular adenosine.

Purinergic control of chemotaxis

A number of reports have shown that addition of exogenous ATP or adenosine can either inhibit or promote neutrophil chemotaxis [110–115]. These contradictory reports can be explained in light of our findings that purinergic signaling systems that are susceptible to ATP and adenosine control several key steps of chemotaxis. We found that the chemoattractant fMLP stimulates the release of ATP from PMN and that this response is greatest at cell membrane areas that are closest to the source of chemoattractant [39]. This suggests that ATP and positive feedback via P2Y2 receptors may be required for gradient sensing. This concept is supported by our findings that scavengers of extracellular ATP, inhibitors of P2 receptors, and the absence of P2Y2 receptors in PMN from P2Y2 knockout (KO) mice render cells unable to orient in a chemotactic gradient field (Fig. 3). ATP and feedback via P2Y2 receptors near sites where ATP is released may constitute a mechanism that significantly amplifies weak chemotactic signal. Chemoattractant receptors and P2Y2 receptors remain uniformly distributed across the cell surface and this does not change after cell polarization [28, 29, 39]. This is critical for PMN, because it allows them to maintain their keen gradient sensing ability while they migrate upstream a chemotactic gradient field.

Once a cell has determined the orientation of the chemotactic gradient field, it rapidly polarizes, assumes an elongated shape, and aligns itself within the chemotactic gradient field. This process involves the accumulation of A3 receptors and likely of ecto-nucleotidases at the leading edge, which allows the conversion of ATP to adenosine. The proximity of adenosine and A3 adenosine receptors at the leading edge allows for a second round of signal amplification. For chemotaxis, this second amplification step is equal in importance to the initial amplification that facilitates gradient sensing. We found that disruption of the latter feedback mechanism with scavengers of extracellular adenosine or A3 adenosine receptor antagonists, or due to the lack of A3 adenosine receptors in PMN from A3 receptor knockout mice, significantly

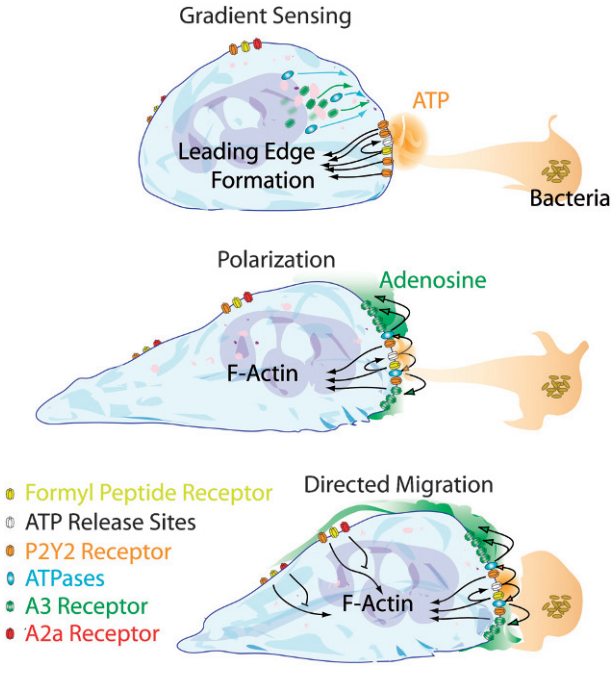

Figure 3. Proposed model of PMN chemotaxis. Formyl peptide receptor (FPR) stimulation causes ATP release from membrane regions closest to the chemotactic source. ATP activates P2Y2 receptors, generating a feedback loop that amplifies chemotactic signals to facilitate gradient sensing. A3 receptors are recruited to the leading edge where ecto-nucleotidases convert ATP to adenosine. Adenosine activates the A3 receptors at the leading edge and A2a receptors at the back of cells, providing local excitation and global inhibition through secondary positive and negative feedback loops that stabilize polarity and promote migration.

impairs chemotaxis. Interference with adenosine signaling significantly reduced migration speed; however, the cells did not lose their ability to detect and correctly orient in the chemotactic gradient field.

The role of A2a receptors in the purinergic control of chemotaxis is less well established. A2a receptors seem to remain uniformly distributed across the cell surface during chemotaxis [39]. This, together with the high affinity of A2a receptors for adenosine and their known suppressive effects on PMN responses, suggests that A2a receptors may participate in controlling PMN chemotaxis. It is likely that A2a receptors fine-tune chemotactic responses by facilitating membrane retraction at the receding edge or by inhibiting chemotactic receptor activation at membrane regions away from the leading edge. Thus, P2Y2 and A3 receptors at the leading edge of PMN seem to facilitate gradient sensing and cell migration, respectively, by activating hallmark signaling molecules associated with front-specific events at the leading edge of polarized cells, while A2a receptors may suppress chemotactic receptor signaling at the trailing edge and thus facilitate membrane retraction at the posterior of cells, where adenosine concentrations are low but sufficient to activate high-affinity A2a adenosine receptors.

Neutrophil abnormalities

The importance of PMN in protecting the host from infections is exemplified by a number of neutrophil abnormalities that severely impair host defense, resulting in recurring infections. PMN dysfunction of chemotaxis may be caused indirectly by the absence of mediators needed to generate chemotactic gradients, for example complement components that form C5a, or by abnormalities of cytoplasmic and granule movement that directly affect chemotactic responses [116–118].

Patients with such disorders are susceptible to infections with a wide spectrum of micro-organisms, including fungi, gram-positive and gram-negative bacteria. *Staphylococcus aureus* infections of the skin, gingival mucosa, and regional lymph nodes are the most frequent bacterial symptoms. Depressed neutrophil chemotaxis has been observed in a wide variety of clinical conditions, including patients suffering from hyperimmunoglobulin E syndrome, Job's syndrome, chronic granulomatous disease, leukocyte adhesion deficiency, and Chediak-Higashi syndrome. At least some of these inherited diseases seem to be associated with defective actin polymerization or granule morphogenesis, with mutation of Rac2, rendering this important signaling molecule unable to bind GTP [119, 120], or with molecular defects that involve CD11 integrins [121, 122]. In most of these diseases, PMN chemotaxis is slowed but PMN remain able to ultimately reach infected sites, suggesting that PMN can compensate, at least in part, for lost functions. Interestingly, knockout mice deficient in either P2Y2 or A3 receptors have defects in the ability to recruit PMN to the peritoneum in response to a septic challenge. However, as in some inherited diseases characterized by defective PMN chemotaxis, P2Y2 and A3 KO mice seemed to compensate for that defect by increasing the numbers of circulating PMN and by increasing the numbers of resident PMN that are dispatched to the peritoneal cavity in healthy animals [39]. Perhaps this is the reason why P2Y2 and A3 KO mice do not appear to suffer from recurrent infectious complications, despite severely impaired PMN chemotaxis.

Apoptosis

PMN apoptosis and the phagocytosis of dying PMN by macrophages are responsible for the clearance of neutrophils from inflamed tissues, which is required for the resolution of acute inflammatory responses [123]. This process is delayed by ATP, which inhibits PMN apoptosis in the presence of growth factors [124]. Adenosine has been shown to either promote or inhibit apoptosis through actions on A3 or A2a adenosine receptors, respectively [125–127]. Interestingly, adenosine is found in synovial fluid samples from patients with rheumatoid arthritis, and the concentrations of adenosine in these samples appear to correlate with inhibitory actions of synovial fluid samples on apoptosis of PMN *in vitro* [128]. However, it is difficult to predict how adenosine influences PMN apoptosis *in vivo* where factors such as a varying fraction of A3 versus A2a receptors expressed on the cell surface of PMN and different adenosine concentrations in microenvironments near the PMN surface may determine the cells' functional responses. Moreover, effector cells such as γδT cells can recognize and kill PMN by direct cell-to-cell interactions, and thus contribute to the clearance of PMN from inflamed host tissues [129]. It is likely that extracellular adenosine and ATP control PMN apoptosis, not only directly but also indirectly by modulating the functions of γδT cells and other cells that are responsible for the clearance of PMN from inflamed sites.

Control of inflammation by ATP and adenosine

Pathological conditions that result in severe cellular distress and cell damage cause ATP release into the extracellular space. For example, hypoxia and tissue injury increase the concentrations of ATP and inflammatory mediators in the circulation in response to hemorrhagic or septic shock. Released ATP is converted to adenosine, which may help prevent excessive phagocyte activation [130]. A large body of evidence suggests that ATP and adenosine could benefit patients with acute inflammatory episodes by modulating functions of immune cells that are involved in the inflammatory response [131]. Indeed, several studies have shown protective effects of infusion of adenosine or ATP-$MgCl_2$, which is rapidly converted to adenosine, in animal models of hemorrhagic or septic shock [131–133]. In addition, ATP and adenosine have been shown to protect lung tissue from ischemia/reperfusion injury [130, 134–137]. Therefore, adenosine is considered an innate protective agent that prevents tissue damage in response to acute or chronic inflammation. Because of the destructive role of PMN in these settings, it is clear that the protective actions of adenosine are, at least in part, due to the suppressive effects of adenosine on PMN. Unfortunately, the clinical use of ATP-$MgCl_2$ and adenosine treatment is hampered by serious side effects that target particularly the cardiovascular system. In attempts to overcome these sequelae, a number of approaches were devised. These approaches induce localized increases in extracellular adenosine concentrations close to the surface of PMN, for example, using inhibitors that target adenosine kinase, adenosine deaminase, or nucleotide transporters [131]. These therapeutic approaches maintain moderately elevated yet consistent levels of adenosine by preventing its phosphorylation, hydrolysis, or cellular re-uptake, respectively. Inhibitors that target these three major processes have also been shown to suppress TNF production in human PMN and to prevent leukocyte adhesion after hemorrhagic shock, endotoxemia, sepsis, colitis, liver ischemia, and reperfusion injury [137–142].

We demonstrated that hypertonic resuscitation fluids can induce the release of ATP from leukocytes, which also results in increased localized concentrations of extracellular adenosine close to the cell surface of PMN. This leads to the suppression of PMN via A2a adenosine receptors [64, 65, 143]. Hypertonic resuscitation fluids were shown to reduce acute inflammatory responses and PMN-induced host tissue injury in a number of animal models of shock [67].

ATP and adenosine regulate many PMN responses including chemotaxis. Therefore, it can be expected that adenosine receptors will be increasingly targeted in future therapeutic approaches to modulate chemotaxis, so as to either reduce PMN recruitment to inflamed tissues or to encourage PMN recruitment to sites of infection.

Adenosine receptors as pharmacological targets to control chemotaxis

All four adenosine receptors are targets of caffeine and related alkaloid drugs widely consumed in the form of tea or coffee. These drugs affect a multitude of physiological processes owing to the abundant expression of the different adenosine receptors in most tissues. A growing body of evidence suggests that pharmacological agents derived from caffeine can be used to target specific adenosine receptor subtypes in various tissues, which would make it possible to treat a number of different conditions including inflammatory diseases. Over the years, highly selective agonists and antagonists of the four adenosine receptor

Table 1. Adenosine receptor subtypes and selected agonists and antagonists.

Receptor subtype	A1 receptor	A2a receptor	A2b receptor	A3 receptor
Official gene name	ADORA1	ADORA2a	ADORA2b	ADORA3
Associated G-proteins	G_i, G_o	G_s, G_{olf}	Gs, G_q	G_i
Selective agonists	CPA CCPA CHA S-ENBA	NECA CGS 21680 HE-NECA CV-1808 CV-1674 ATL146e	LUF5835	IB-MECA Cl-IB-MECA MRS1898
Selective antagonists	DPCPX WRC0571 BG9719 FK453	VER6947 VER7835 SCH58261. ZM241385	MRS1754 MRE2029-F20	MRS1220 MRE3008-F20 MRS1191 MRS1523

subtypes have been developed [144, 145]. Table 1 summarizes some of these agents.

In the context of this review, drugs that affect A2a and A3 adenosine receptors are of particular interest because the receptors are highly expressed in PMN and appear to play key roles in controlling PMN chemotaxis. We found that MRS 1191, an A3 receptor antagonist, impairs chemotaxis of human PMN *in vitro* by inhibiting the rate of migration [39], while the A2a receptor agonist CGS 21680 appears to reduce chemotaxis by affecting retraction of the receding edge (unpublished data). *In vivo* studies with A3 receptor deficient mice support the concept that inhibition or elimination of A3 adenosine receptors may prove useful to reduce PMN chemotaxis in response to inflammatory challenges [39]. Agonists of A3 receptors enhance PMN chemotaxis *in vitro* [39]. Interestingly, leukocytes of rheumatoid arthritis patients have been found to over-express A3 adenosine receptors, suggesting that increased A3 receptor expression could be related to the accumulation of PMN in inflamed joints [146]. Taken together, these findings imply that A3 receptors are potential pharmacological targets for novel therapies aimed at stemming the inflammatory response, for example, in rheumatoid arthritis [147, 148].

Ample evidence suggests that A2a receptor agonists, which inhibit PMN functions, including chemotaxis, by increasing intracellular cAMP levels, may also prove useful to prevent PMN accumulation and tissue damage in a number of inflammatory complications, particularly chronic airway inflammation and related respiratory disorders [149–151].

The potential therapeutic value of drugs that target A3 receptors may not be limited to inhibiting PMN chemotaxis, as A3 adenosine receptors may also play a role in the migration of other cell types, possibly including cancer cells. Thus, pharmacological targeting of A3 receptors could find use in treating certain types of cancer, a hypothesis that is strengthened by preclinical findings that chronic use of A3 receptor agonists may be useful for the treatment of breast and colon cancers, and by the observation that colorectal cancer is associated with elevated A3 receptor expression [152–154].

The underlying mechanisms of action are not fully understood, but it is possible that chronic use of A3 receptor agonists may either cause receptor desensitization, receptor internalization, or simply interfere with chemotaxis processes by obscuring endogenous adenosine gradients that drive directed cell migration. Although the results of preclinical studies suggest that drugs that target adenosine receptors may be efficacious to treat a variety of diseases such as cancer, arthritis, and a host of other inflammatory diseases, none of these drugs has yet received regulatory approval. However, this may change as our understanding of the complex mechanisms that govern the expression and activation of adenosine receptors in different host tissues improves due to the relentless efforts of many experts in the respective research fields.

Acknowledgements. This study was partially supported by the National Institute of General Medical Sciences Grants R01 GM-51477, GM-60475, and CDMRP Grant PR043034.

1 Segal,, A. W. (2006) How superoxide production by neutrophil leukocytes kills microbes. Novartis Found Symp. 279, 92–98 .
2 Nauseef,, W. M. (2007) How human neutrophils kill and degrade microbes: an integrated view. Immunol. Rev. 219, 88–102.
3 Brinkmann,, V. and Zychlinsky,, A. (2007) Beneficial suicide: why neutrophils die to make NETs. Nat. Rev. Microbiol. 5, 577–582.
4 Chaudry,, I. H. and Ayala,, A. (1992) Immunological aspects of hemorrhage, pp 1–132, RG Landes Company, Austin Texas.
5 Botha, A. J., Moore, F. A., Moore, E. E., Sauaia, A., Banerjee, A. and Peterson, V. M. (1995) Early neutrophil sequestration after injury: a pathogenic mechanism for multiple organ failure. J. Trauma 39, 411–417.
6 Fujishima, S. and Aikawa, N. (1995) Neutrophil-mediated tissue injury and its modulation. Intensive Care Med. 21, 277–285.

7 Reutershan, J. and Ley, K. (2004) Bench-to-bedside review: acute respiratory distress syndrome – how neutrophils migrate into the lung. Crit. Care 8, 453–461.
8 Baker, C. C., Oppenheimer, L., Lewis, F. R. and Trunkey, D. D. (1980) The epidemiology of trauma death. Am. J. Surg. 140, 144–150.
9 Hoyt, D. B., Bulger, E. M., Knudson, M. M. Morris, J., Ierardi, R., Sugerman, H. J., Shackford, S. R., Landercasper, J., Winchell, R. J., Jurkovich, G. (1994) Death in the operating room: an analysis of a multi-center experience. J. Trauma 37, 426–432.
10 Sauaia, A., Moore, F. A., Moore, E. E., Moser, K. S., Brennan, R., Read, R. A. and Pons, P. T. (1995) Epidemiology of trauma deaths: a reassessment. J. Trauma 38, 185–193.
11 Seely, A. J., Pascual, J. L. and Christou, N. V. (2003) Science review: Cell membrane expression (connectivity) regulates neutrophil delivery, function and clearance. Crit. Care 7, 291–307.
12 Cazzola, M., Ciaprini, C., Page, C. P. and Matera, M. G. (2007) Targeting systemic inflammation: novel therapies for the treatment of chronic obstructive pulmonary disease. Expert. Opin. Ther. Targets 11, 1273–1286.
13 Bosma, K. J. and Lewis, J. F. (2007) Emerging therapies for treatment of acute lung injury and acute respiratory distress syndrome. Expert Opin. Emerg. Drugs. 12, 461–477.
14 Rubin, B. K. and Fink, J. B. (2007) Novel medications for asthma: a look at the future. Expert. Opin. Investig. Drugs 16, 889–897.
15 Luster, A. D., Alon, R. and von Andrian, U. H. (2005) Immune cell migration in inflammation: present and future therapeutic targets. Nat. Immunol. 6, 1182–1190.
16 Kondo, M., Wagers, A. J., Manz, M. G., Prohaska, S. S., Scherer, D. C., Beilhack, G. F., Shizuru, J. A. and Weissman, I. L. (2003) Biology of hematopoietic stem cells and progenitors: Implications for clinical application. Annu. Rev. Immunol. 21, 759–806.
17 Spangrude, G. J. (2003) When is a stem cell really a stem cell? Bone Marrow Transplant 32 (Suppl 1), S7.
18 Smaaland, R., Sothern, R. B., Laerum, O. D. and Abrahamsen, J. F. (2002) Rhythms in human bone marrow and blood cells. Chronobiol. Int. 19, 101–127.
19 Metcalf, D. (2001) Hematopoietic stem cells: Old and new. Biomed. Pharmacother. 55, 75–78.
20 Metcalf, D. (1988) Colony stimulating factors and hemopoiesis. Ann. Acad. Med. Singapore 17, 166–170.
21 Stark, M. A., Huo, Y., Burcin, T. L., Morris, M. A., Olson, T. S. and Ley, K. (2005) Phagocytosis of apoptotic neutrophils regulates granulopoiesis via IL-23 and IL-17. Immunity 22, 285–294.
22 Bunting, M., Harris, E. S., McIntyre, T. M., Prescott, S. M. and Zimmerman, G. A. (2002) Leukocyte adhesion deficiency syndromes: adhesion and tethering defects involving beta 2 integrins and selectin ligands. Curr. Opin. Hematol. 9, 30–35.
23 Yonekawa, K. and Harlan, J. M. (2004) Targeting leukocyte integrins in human diseases. J. Leukoc. Biol. 77, 129–140.
24 Engelhardt, B. and Wolburg, H. (2004) Mini-review: Transendothelial migration of leukocytes: through the front door or around the side of the house? Eur. J. Immunol. 34, 2955–2963.
25 Petri, B. and Bixel, M. G. (2006) Molecular events during leukocyte diapedesis. FEBS J. 273, 4399–4407.
26 McIntyre, T. M., Prescott, S. M., Weyrich, A. S. and Zimmerman, G. A. (2003) Cell-cell interactions: leukocyte-endothelial interactions. Curr. Opin. Hematol. 10, 150–158.
27 Eddy, R. J., Pierini, L. M., Matsumura, F. and Maxfield, F. R. (2000) Ca2+-dependent myosin II activation is required for uropod retraction during neutrophil migration. J. Cell Sci. 113, 1287–1298.
28 Pollard, T. D. and Borisy, G. G. (2003) Cellular motility driven by assembly and disassembly of actin filaments. Cell 112, 453–465.
29 Xu, J., Wang, F., Van Keymeulen, A., Herzmark, P., Straight, A., Kelly, K., Takuwa, Y., Sugimoto, N., Mitchison, T. and Bourne, H. R. (2003) Divergent signals and cytoskeletal assemblies regulate self-organizing polarity in neutrophils. Cell 114, 201–214.
30 Van Haastert, P. J. and Devreotes, P. N. (2004) Chemotaxis: signaling the way forward. Nat. Rev. Mol. Cell Biol. 5, 626–634.
31 Weiner, O. D., Neilsen, P. O., Prestwich, G. D., Kirschner, M. W., Cantley, L. C. and Bourne, H. R. (2002) A PtdInsP(3)- and Rho GTPase-mediated positive feedback loop regulates neutrophil polarity. Nat. Cell Biol. 4, 509–513.
32 Franca-Koh, J. and Devreotes, P. N. (2004) Moving forward: mechanisms of chemoattractant gradient sensing. Physiology (Bethesda) 19, 300–308.
33 Franca-Koh, J., Kamimura, Y. and Devreotes, P. (2006) Navigating signaling networks: chemotaxis in Dictyostelium discoideum. Curr. Opin. Genet. Dev. 16, 333–338.
34 Charest, P. G. and Firtel, R. A. (2006) Feedback signaling controls leading-edge formation during chemotaxis. Curr. Opin. Genet. Dev. 16, 339–347.
35 Meinhardt, H. (1999) Orientation of chemotactic cells and growth cones: models and mechanisms. J. Cell Sci. 112, 2867–2874.
36 Meinhardt, H. and Gierer, A. (2000) Pattern formation by local self-activation and lateral inhibition. Bioessays 22, 753–760.
37 Levchenko, A. and Iglesias, P. A. (2002) Models of eukaryotic gradient sensing: application to chemotaxis of amoebae and neutrophils. Biophys. J. 82, 50–63.
38 Ma, L., Janetopoulos, C., Yang, L., Devreotes, P. N. and Iglesias, P. A. (2004) Two complementary, local excitation, global inhibition mechanisms acting in parallel can explain the chemoattractant-induced regulation of PI(3,4,5)P3 response in Dictyostelium cells. Biophys. J. 87, 3764–3774.
39 Chen, Y., Corriden, R., Inoue, Y., Yip, L., Hashiguchi, N., Zinkernagel, A., Nizet, V., Insel, P. A. and Junger, W. G. (2006) ATP release guides neutrophil chemotaxis via P2Y2 and A3 receptors. Science, 314, 1792–1795.
40 Abbracchio, M. P., Burnstock, G., Boeynaems, J. M., Barnard, E. A., Boyer, J. L., Kennedy, C., Knight, G. E., Fumagalli, M., Gachet, C., Jacobson, K. A. and Weisman, G. A. (2006) International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. Pharmacol. Rev. 58, 281–341.
41 Mo, F. M. and Ballard, H. J. (2001) The effect of systemic hypoxia on interstitial and blood adenosine, AMP, ADP and ATP in dog skeletal muscle. J. Physiol. 536, 593–603.
42 Vassort, G. (2001) Adenosine 5-triphosphate: a P2-purinergic agonist in the myocardium. Physiol. Rev. 81, 767–806.
43 Ralevic, V. and Burnstock, G. (1998) Receptors for purines and pyrimidines. Pharmacol. Rev. 50, 413–492.
44 Milner, P., Bodin, P., Loesch, A. and Burnstock, G. (1990) Rapid release of endothelin and ATP from isolated aortic endothelial cells exposed to increased flow. Biochem. Biophys. Res. Commun. 170, 649–656.
45 Bodin, P., Bailey, D. and Burnstock, G. (1991) Increased flow-induced ATP release from isolated vascular endothelial but not smooth muscle cells. Br. J. Pharmacol. 103, 1203–1205.
46 Pedersen, S., Pedersen, S. F., Nilius, B., Lambert, I. H. and Hoffmann, E. K. (1999) Mechanical stress induces release of ATP from Ehrlich ascites tumor cells. Biochim. Biophys. Acta 1416, 271–284.
47 Yegutkin, G., Bodin, P. and Burnstock, G. (2000) Effect of shear stress on the release of soluble ecto-enzymes ATPase and 5'-nucleotidase along with endogenous ATP from vascular endothelial cells. Br. J. Pharmacol. 129, 921–926.
48 Ostrom, R. S., Gregorian, C., Drenan, R. M., Gabot, K., Rana, B. K. and Insel, P. A. (2001) Key role for constitutive cyclooxygenase-2 of MDCK cells in basal signaling and

response to released ATP. Am. J. Physiol. Cell Physiol. 281, C524-C531.
49 Ostrom, R. S., Gregorian, C. and Insel, P. A. (2000) Cellular release of and response to ATP as key determinants of the set-point of signal transduction pathways. J. Biol. Chem. 275, 11735–11739.
50 Schwiebert, E. M. and Zsembery, A. (2003) Extracellular ATP as a signaling molecule for epithelial cells. Biochim. Biophys. Acta 1615, 7–32.
51 Novak, I. (2003) ATP as a signaling molecule: the exocrine focus. News Physiol. Sci. 18, 12–17.
52 Sabirov, R. Z. and Okada, Y. (2004) ATP-conducting maxi-anion channel: A new player in stress-sensory transduction. Jpn. J. Physiol. 54, 7–14.
53 Silinsky, E. M. (1975) On the association between transmitter secretion and the release of adenine nucleotides from mammalian motor nerve terminals. J. Physiol. 247, 145–162.
54 Zimmermann, H. (1994) Signaling via ATP in the nervous system. Trends Neurosci. 17, 420–426.
55 Jo, Y. H. and Schlichter, R. (1999) Synaptic corelease of ATP and GABA in cultured spinal neurons. Nat. Neurosci. 2, 241–245.
56 Hazama, A., Fan, H. T., Abdullaev, I., Maeno, E., Tanaka, S., Ando-Akatsuka, Y. and Okada, Y. (2000) Swelling-activated, cystic fibrosis transmembrane conductance regulator augmented ATP release and Cl- conductances in murine C127 cells. J. Physiol. 523, 1–11.
57 Osipchuk, Y. and Cahalan, M. (1992) Cell-to-cell spread of calcium signals mediated by ATP receptors in mast cells. Nature 359, 241–244.
58 Unsworth, C. D. and Johnson, R. G. (1990) Acetylcholine and ATP are coreleased from the electromotor nerve terminals of Narcine brasiliensis by an exocytotic mechanism. Proc. Natl. Acad. Sci. USA 87, 553–557.
59 Musante, L., Zegarra-Moran, O., Montaldo, P. G., Ponzoni, M. and Galietta, L. J. (1999) Autocrine regulation of volume-sensitive anion channels in airway epithelial cells by adenosine. J. Biol. Chem. 274, 11701–11707.
60 Van der Wijk, T., De Jonge, H. R. and Tilly, B. C. (1999) Osmotic cell swelling-induced ATP release mediates the activation of extracellular signal-regulated protein kinase (Erk)-1/2 but not the activation of osmo-sensitive anion channels. Biochem. J. 343, 579–586.
61 Sauer, H., Hescheler, J. and Wartenberg, M. (2000) Mechanical strain-induced Ca2+ waves are propagated via ATP release and purinergic receptor activation. Am. J. Physiol. 279, C295-C307.
62 Koyama, T., Oike, M. and Ito, Y. (2001) Involvement of Rho-kinase and tyrosine kinase in hypotonic stress-induced ATP release in bovine aortic endothelial cells. J. Physiol. 532, 759–769.
63 Loomis, W. H., Namiki, S., Ostrom, R. S., Insel, P. A. and Junger, W. G. (2003) Hypertonic stress increases T cell interleukin-2 expression through a mechanism that involves ATP release, P2 receptor, and p38 MAPK activation. J. Biol. Chem. 278, 4590–4596.
64 Chen, Y., Shukla, A., Namiki, S., Insel, P. A. and Junger, W. G. (2004) A putative osmoreceptor system that controls neutrophil function through the release of ATP, its conversion to adenosine, and activation of A2 adenosine and P2 receptors. J. Leukoc. Biol. 76, 245–253.
65 Chen, Y., Hashiguchi, N., Yip, L. and Junger WG. (2006) Hypertonic saline enhances neutrophil elastase release through activation of P2 and A3 receptors. Am. J. Physiol. Cell Physiol. 290, C1051- C1059.
66 Yip, L., Cheung, C. W., Corriden, R., Chen, Y., Insel, P. A. and Junger, W. G. (2007) Hypertonic stress regulates T-cell function by the opposing actions of extracellular adenosine triphosphate and adenosine. Shock 27, 242–250.
67 Shukla, A., Hashiguchi, N., Chen, Y., Coimbra, R., Hoyt, D. B. and Junger, W. G. (2004) Osmotic regulation of cell function and possible clinical applications. Shock 21, 391–400.
68 Perel, P. and Roberts, I. (2007) Colloids versus crystalloids for fluid resuscitation in critically ill patients. Cochrane Database Syst. Rev. 17, CD000567.
69 Himmelseher, S.(2007) Hypertonic saline solutions for treatment of intracranial hypertension. Curr. Opin. Anaesthesiol. 20, 414–426.
70 Tyagi, R., Donaldson, K., Loftus, C. M. and Jallo, J. (2007) Hypertonic saline: a clinical review. Neurosurg. Rev., 30, 277–289.
71 Eltzschig, H. K., Eckle, T., Mager, A., Küper, N., Karcher, C., Weissmüller, T., Boengler, K., Schulz, R., Robson, S. C. and Colgan, S. P. (2006) ATP release from activated neutrophils occurs via connexin 43 and modulates adenosine-dependent endothelial cell function. Circ. Res. 99, 1100–1108.
72 Corriden, R., Insel, P. A. and Junger, W. G. (2007) A novel method using fluorescence microscopy for real-time assessment of ATP release from individual cells. Am. J. Physiol. Cell Physiol. 293, C1420-C1425.
73 Sabirov, R. Z., Dutta, A. K. and Okada, Y. (2001) Volume-dependent ATP-conductive large-conductance anion channel as a pathway for swelling-induced ATP release. J. Gen. Physiol. 118, 251–266..
74 McCann, F. V., McCarthy, D. C., Keller, T. M. and Noelle, R. J. (1989) Characterization of a large conductance non-selective anion channel in B lymphocytes. Cell. Signal. 1, 31–44.
75 Schlichter, L. C., Grygorczyk, R., Pahapill, P. A. and Grygorczyk, C. (1990) A large, multiple-conductance chloride channel in normal human T lymphocytes. Pflugers Arch. 416, 413–421.
76 Pahapill, P. A. and Schlichter, L. C. (1992) Cl- channels in intact human T lymphocytes. J. Membr. Biol. 125, 171–183.
77 Bell, P. D., Lapointe, J. Y., Sabirov, R., Hayashi, S., Peti-Peterdi, J., Manabe, K., Kovacs, G. and Okada, Y. (2003) Macula densa cell signaling involves ATP release through a maxi anion channel. Proc. Natl. Acad. Sci. USA 100, 4322–4327.
78 Komlosi, P., Fintha, A. and Bell, P. D. (2005) Renal cell-to-cell communication via extracellular ATP. Physiology (Bethesda) 20, 86–90.
79 Suzuki, M. and Mizuno, A. (2004) A novel human Cl- channel family related to Drosophila flightless locus. J. Biol. Chem. 279, 22461–22468.
80 Suzuki, M., Sato, J., Kutsuwada, K., Ooki, G. and Imai, M. (1999) Cloning of a stretch-inhibitable nonselective cation channel. J. Biol. Chem. 274, 6330–6335.
81 Lee, J., Gustafsson, M., Magnusson, K. E. and Jacobson, K. (1990) The direction of membrane lipid flow in locomoting polymorphonuclear leukocytes. Science 247, 1229–1233.
82 Marks, P. W. and Maxfield, F. R. (1990) Local and global changes in cytosolic free calcium in neutrophils during chemotaxis and phagocytosis. Cell Calcium 11, 181–190.
83 Zarbock, A., Polanowska-Grabowska, R. K. and Ley, K. (2007) Platelet-neutrophil-interactions: linking hemostasis and inflammation. Blood Rev. 21, 99–111.
84 Fredholm, B. B. (1997) Purines and neutrophil leukocytes. Gen. Pharmacol. Gen. Pharmacol. 28, 345–350.
85 Bours, M. J., Swennen, E. L., Di Virgilio, F., Cronstein, B. N. and Dagnelie, P. C. (2006) Adenosine 5'-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. Pharmacol. Ther. 112, 358–404.
86 Elia, M. G., Muscella, A., Romano, S., Greco, S., Di Jeso, B., Verri, T., Storelli, C. and Marsigliante, S. (2005) Effects of extracellular nucleotides in the thyroid: P2Y2 receptor-mediated ERK1/2 activation and c-Fos induction in PC Cl3 cells. Cell Signal. 17, 739–749.
87 Santiago-Perez, L. I., Flores, R. V., Santos-Berrios, C., Chorna, N. E., Krugh, B., Garrad, R. C., Erb, L., Weisman, G. A. and Gonzalez, F. A. (2001) P2Y(2) nucleotide receptor signaling in human monocytic cells: activation, desensitization and coupling to mitogen-activated protein kinases. J. Cell Physiol. 187, 196–208.

88 Merighi, S., Benini, A., Mirandola, P., Gessi, S., Varani, K., Leung, E., Maclennan, S. and Borea, P. A. (2005) A3 adenosine receptor activation inhibits cell proliferation via phosphatidylinositol 3-kinase/Akt-dependent inhibition of the extracellular signal-regulated kinase 1/2 phosphorylation in A375 human melanoma cells. J. Biol. Chem. 280, 19516–19526.

89 Hammarberg, C., Fredholm, B. B. and Schulte, G. (2004) Adenosine A3 receptor-mediated regulation of p38 and extracellular-regulated kinase ERK1/2 via phosphatidylinositol-3'-kinase. Biochem. Pharmacol. 67, 129–134.

90 Jin, J., Dasari, V. R., Sistare, F. D. and Kunapuli, S. P. (1998) Distribution of P2Y receptor subtypes on haematopoietic cells. Br. J. Pharmacol. 123, 789–794.

91 Di Virgilio, F., Borea, P. A. and Illes, P. (2001) P2 receptors meet the immune system. Trends Pharmacol. Sci. 22, 5–7.

92 Di Virgilio, F., Chiozzi, P., Ferrari, D., Falzoni, S., Sanz, J. M., Morelli, A., Torboli, M., Bolognesi, G. and Baricordi, O. R. (2001) Nucleotide receptors: an emerging family of regulatory molecules in blood cells. Blood 97, 587–600.

93 Robson, S. C., Sevigny, J. and Zimmermann, H. (2006) The E-NTPDase family of ecto-nucleotidases: Structure function relationships and pathophysiological significance. Purinergic Signaling 2, 409–430.

94 Robson, S. C., Wu, Y., Sun, X., Knosalla, C., Dwyer, K. and Enjyoji, K. (2005) Ectonucleotidases of CD39 family modulate vascular inflammation and thrombosis in transplantation. Semin. Thromb. Hemost. 31, 217–233.

95 Beldi, G., Enjyoji, K., Wu, Y., Miller, L., Banz, Y., Sun, X. and Robson, S. C. (2008) The role of purinergic signaling in the liver and in transplantation: effects of extracellular nucleotides on hepatic graft vascular injury, rejection and metabolism. Front Biosci. 13, 2588–2603.

96 Zimmermann, H. (2000) Extracellular metabolism of ATP and other nucleotides. Naunyn-Schmiedeberg's Arch. Pharmacol. 362, 299–309.

97 Zimmermann, H. (2001) Some recent developments and a note on nomenclature. Drug Dev. Res. 52, 44–56.

98 Zimmermann, H. (2006) Ectonucleotidases in the nervous system. Novartis Found. Symp. 276, 113–128.

99 Pulte, E. D., Broekman, M. J., Olson, K. E., Drosopoulos, J. H., Kizer, J. R., Islam, N. and Marcus, A. J. (2007) CD39/NTPDase-1 activity and expression in normal leukocytes. Thromb. Res. 121, 309–317.

100 Koziak, K., Sévigny, J., Robson, S. C., Siegel, J. B. and Kaczmarek, E. (1999) Analysis of CD39/ATP diphosphohydrolase (ATPDase) expression in endothelial cells, platelets and leukocytes. Thromb. Haemost. 82, 1538–1544.

101 Wilson, P. D., Rustin, G. J., Smith, G. P. and Peters, T. J. (1981) Electron microscopic cytochemical localization of nucleoside phosphatases in normal and chronic granulocytic leukaemic human neutrophils. Histochem. J. 13, 73–84.

102 Stewart, D. I. AND Crawford, N. (1983) Redistribution of membrane 5'-nucleotidase in rabbit peritoneal polymorphonuclear leucocytes during phagocytosis. FEBS Lett. 156, 329–334.

103 Cramer, E., Werb, Z. and Bainton, D. F. (1984) Redistribution of ecto-5'-nucleotidase during phagocytosis by guinea pig polymorphonuclear leukocytes. J. Leukoc. Biol. 36, 463–475.

104 Kitakaze, M., Hori, M., Morioka, T., Takashima, S., Minamino, T., Sato, H., Inoue, M. and Kamada, T. (1993) Attenuation of ecto-5'-nucleotidase activity and adenosine release in activated human polymorphonuclear leukocytes. Circ. Res. 73, 524–533.

105 Sato, N., Takahashi, Y. and Asano, S. (1994) Preferential usage of the bone-type leader sequence for the transcripts of liver/bone/kidney-type alkaline phosphatase gene in neutrophilic granulocytes. Blood 83, 1093–1101.

106 Shibano, M., Machii, T., Nishimori, Y., Nakamoto, I., Ueda, E., Masuhara, K. and Kitani, T. (1999) Assessment of alkaline phosphatase on the surface membrane of neutrophils by immunofluorescence. Am. J. Hematol. 60, 12–8.

107 Heyneman, R. and Burvenich, C. (1992) Kinetics and characteristics of bovine neutrophil alkaline phosphatase during acute Escherichia coli mastitis. J. Dairy Sci. 75, 1826–1834.

108 Karlsson, A., Khalfan, L., Dahlgren, C., Stigbrand, T. and Follin, P. (1995) Neutrophil alkaline phosphatase activity increase in bacterial infections is not associated with a general increase in secretory vesicle membrane components. Infect. Immun. 63, 911–916.

109 Laharrague, P., Denier, C. J., Grozdea, J., Perie, J. J., Corberand, J. X., Fillola, G. and Vergnes, H. (1998) Atypical neutrophil alkaline phosphatase associated with impaired neutrophil functions. Clin. Chim. Acta 277, 77–89.

110 Elferink, J. G., de Koster, B. M., Boonen, G. J. and de Priester, W. (1992) Inhibition of neutrophil chemotaxis by purinoceptor agonists. Arch. Int. Pharmacodyn. Ther. 317, 93–106.

111 Verghese, M. W., Kneisler, T. B. and Boucheron, J. A. (1996) P2U agonists induce chemotaxis and actin polymerization in human neutrophils and differentiated HL60 cells. J. Biol. Chem. 271, 15597–15601.

112 Kaneider, N. C., Mosheimer, B., Reinisch, N., Patsch, J. R. and Wiedermann, C. J. (2004) Inhibition of thrombin-induced signaling by resveratol and quercetin: effects on adenosine nucleotide metabolism in endothelial cells and platelet–neutrophil interactions. Thromb. Res. 144, 185–194.

113 Rose, F. R., Hirschhorn, R., Weissmann, G. and Cronstein, B. N. (1988) Adenosine promotes neutrophil chemotaxis. J. Exp. Med. 167, 1186–1194.

114 Cronstein, B. N., Daguma, L., Nichols, D., Hutchison, A. J. and Williams, M. (1990) The adenosine/neutrophil paradox resolved: human neutrophils possess both A1 and A2 receptors that promote chemotaxis and inhibit O2 generation, respectively. J. Clin. Invest. 85, 1150–1157.

115 Bouma, M. G., van den Wildenberg, F. A. and Buurman, W. A. (1996) Adenosine inhibits cytokine release and expression of adhesion molecules by activated human endothelial cells, Am. J. Physiol. 270, C522–C529.

116 Lekstrom-Himes, J. A. and Gallin, J. I. (2000) Immunodeficiency diseases caused by defects in phagocytes., N. Engl. J. Med. 343, 1703–1714.

117 Andrews, T. and Sullivan, K. E. (2003) Infections in patients with inherited defects in phagocytic function. Clin. Microbiol. Rev. 16, 597–621.

118 Lakshman, R. and Finn, A. (2001) Neutrophil disorders and their management. J. Clin. Pathol. 54, 7–19.

119 Ambruso, D. R., Knall, C., Abell, A. N., Panepinto, J., Kurkchubasche, A., Thurman, G., Gonzalez-Aller, C., Hiester, A., deBoer, M., Harbeck, R. J., Oyer, R., Johnson, G. L. and Roos, D. (2000) Human neutrophil immunodeficiency syndrome is associated with an inhibitory Rac2 mutation. Proc. Natl. Acad. Sci. USA 97, 4654–4659.

120 Williams, D. A., Tao, W., Yang, F., Kim, C., Gu, Y., Mansfield, P., Levine, J. E., Petryniak, B., Derrow, C. W., Harris, C., Jia, B., Zheng, Y., Ambruso, D. R., Lowe, J. B., Atkinson, S. J., Dinauer, M. C. and Boxer, L. (2000) Dominant negative mutation of the hematopoietic-specific Rho GTPase, Rac2, is associated with a human phagocyte immunodeficiency. Blood 96, 1646–1654.

121 Boxer, L. A. and Smolen, J. E. (1988) Neutrophil granule constituents and their release in health and disease. Hematol. Oncol. Clin. North Am. 2, 101–134.

122 Ward, D. M., Shiflett, S. L. and Kaplan, J. (2002) Chediak-Higashi syndrome: A clinical and molecular view of a rare lysosomal storage disorder. Curr. Mol. Med. 2, 469–477.

123 Simon, H. U. (2003) Neutrophil apoptosis pathways and their modifications in inflammation, Immunol. Rev. 193, 101–110.

124 Gasmi, L., McLennan, A. G. and Edwards, S. W. (1996) The diadenosine polyphosphates Ap3A and Ap4A and adenosine triphosphate interact with granulocyte-macrophage colony-stimulating factor to delay neutrophil apoptosis: implications for neutrophil: platelet interactions during inflammation. Blood 87, 3442–3449.

125 Kohno, Y., Sei, Y., Koshiba, M., Kim, H. O. and Jacobson, K. A. (1996) Induction of apoptosis in HL-60 human promyelocytic leukemia cells by adenosine A(3) receptor agonists. Biochem. Biophys. Res. Commun. 219, 904–910.
126 Walker, B. A., Rocchini, C., Boone, R. H.,, R. H. Ip, R. H. and Jacobson, M. A. (1997) Adenosine A2a receptor activation delays apoptosis in human neutrophils. J. Immunol. 158, 2926–2931.
127 Yasui, K., Agematsu, K., Shinozaki, K., Hokibara, S., Nagumo, H., Nakazawa, T. and Komiyama, A. (2000) Theophylline induces neutrophil apoptosis through adenosine A2A receptor antagonism. J. Leukoc. Biol. 67, 529–535.
128 Ottonello, L., Cutolo, M., Frumento, G., Arduino, N., Bertolotto, M., Mancini, M., Sottofattori, E. and Dallegri, F. (2004) Synovial fluid from patients with rheumatoid arthritis inhibits neutrophil apoptosis: role of adenosine and proinflammatory cytokines. Rheumatology (Oxford) 41, 1249–1260.
129 Hirsh, M. I., Hashiguchi, N., Chen, Y., Yip, L. and Junger, W. G. (2006) Surface expression of HSP72 by LPS-stimulated neutrophils facilitates gammadelta T cell-mediated killing. Eur. J. Immunol. 36, 712–721.
130 Bouma, M. G., van den Wildenberg, F. A. and Buurman, W. A. (1997) The anti-inflammatory potential of adenosine in ischemia-reperfusion injury: established and putative beneficial actions of a retaliatory metabolite. Shock 8, 313–320.
131 McCallion, K., Harkin, D. W. and Gardiner, K. R. (2004) Role of adenosine in immunomodulation: Review of the literature. Crit. Care Med. 32, 273–277.
132 Chaudry, I. H., Sayeed, M. M. and Baue, A. E. (1974) Effect of adenosine triphosphate-magnesium chloride administration in shock. Surgery 75, 220–227.
133 Nalos, M., Asfar, P., Ichai, C., Radermacher, P., Leverve, X. M. and Froba, G. (2003) Adenosine triphosphate-magnesium chloride: relevance for intensive care. Intensive Care Med 29, 10–18.
134 Harkema, J. M. and Chaudry, I. H. (1992) Magnesium-adenosine triphosphate in the treatment of shock, ischemia, and sepsis. Crit. Care Med. 20, 263–275.
135 Firestein, G. S., Boyle, D., Bullough, D. A., Gruber, H. E., Sajjadi, F. G., Montag, A., Sambol, B. and Mullane, K. M. (1994) Protective effect of an adenosine kinase inhibitor in septic shock. J. Immunol. 152, 5853–5859.
136 Bullough, D. A., Magill, M. J., Firestein, G. S. and Mullane, K. M. (1995) Adenosine activates A2 receptors to inhibit neutrophil adhesion and injury to isolated cardiac myocytes. J. Immunol. 155, 2579–2586.
137 Eigler, A., Matschke, V., Hartmann, G., Erhardt, S., Boyle, D., Firestein, G. S. and Endres, S. (2000) Suppression of TNF-alpha production in human mononuclear cells by an adenosine kinase inhibitor. J. Leukoc. Biol. 68, 97–103.
138 Bauer, C., Bouma, M. G., Herrmann, I., van den Wildenberg, F. A., Firestein, G. S., Marzi, I. and Buurman, W. A. (1997) Adenosine kinase inhibitor GP515 attenuates hepatic leukocyte adhesion after hemorrhagic hypotension. Am. J. Physiol. 273, G1297–1303.
139 Adanin, S., Yalovetskiy, I. V., Nardulli, B. A., Sam, A. D. 2nd, Jonjev, Z. S. and Law, W. R. (2002) Inhibiting adenosine deaminase modulates the systemic inflammatory response syndrome in endotoxemia and sepsis. Am. J. Physiol. Regul. Integr. Comp. Physiol. 282, R1324- R1332.
140 Siegmund, B., Rieder, F., Albrich, S., Wolf, K., Bidlingmaier, C., Firestein, G. S., Boyle, D., Lehr, H. A., Loher, F., Hartmann, G., Endres, S. and Eigler, A. (2001) Adenosine kinase inhibitor GP515 improves experimental colitis in mice. J. Pharmacol. Exp. Ther. 296, 99–105.
141 Todo, S., Zhu, Y., Zhang, S., Jin, M. B., Ishizaki, N., Tanaka, H., Subbotin, V. and Starzl, T. E. (1997) Attenuation of ischemic liver injury by augmentation of endogenous adenosine. Transplantation 63, 217–223.
142 Schoenberg, M. H., Fredholm, B. B., Haglund, U., Jung, H., Sellin, D., Younes, M. and Schildberg, F. W. (1985) Studies on the oxygen radical mechanism involved in the small intestinal reperfusion damage. Acta Physiol. Scand. 124, 581–589.
143 Junger, W. G., Hoyt, D. B., Davis, R. E., Herdon-Remelius, C., Namiki, S., Junger, H., Loomis, W. and Altman, A. (1998) Hypertonicity regulates the function of human neutrophils by modulating chemoattractant receptor signaling and activating mitogen-activated protein kinase p38. J. Clin. Invest. 101, 2768–2779.
144 Jacobson, K. A. and Gao, Z. G. (2006) Adenosine receptors as therapeutic targets. Nat. Rev. Drug Discov. 5, 247–264.
145 Fredholm, B. B. (2007) Adenosine, an endogenous distress signal, modulates tissue damage and repair. Cell Death Differ. 14, 1315–1323.
146 Madi, L., Cohen, S., Ochayin, A., Bar-Yehuda, S., Barer, F. and Fishman, P. (2007) Overexpression of A3 adenosine receptor in peripheral blood mononuclear cells in rheumatoid arthritis: involvement of nuclear factor-kappaB in mediating receptor level. J. Rheumatol. 34, 20–26.
147 Bar-Yehuda, S., Silverman, M. H., Kerns, W. D., Ochaion, A., Cohen, S. and Fishman, P. (2007) The anti-inflammatory effect of A3 adenosine receptor agonists: a novel targeted therapy for rheumatoid arthritis. Expert. Opin. Investig. Drugs 16, 1601–1613.
148 Fishman, P. and Bar-Yehuda, S. (2003) Pharmacology and therapeutic applications of A3 receptor subtype. Curr. Top. Med. Chem. 3, 463–469.
149 Polosa, R. and Holgate, S. T. (2006) Adenosine receptors as promising therapeutic targets for drug development in chronic airway inflammation. Curr. Drug Targets 7, 699–706.
150 McColl, S. R., St-Onge, M., Dussault, A. A., Laflamme, C., Bouchard, L., Boulanger, J. and Pouliot, M. (2006) Immunomodulatory impact of the A2A adenosine receptor on the profile of chemokines produced by neutrophils. FASEB J. 20, 187–189.
151 Bonneau, O., Wyss, D., Ferretti, S., Blaydon, C., Stevenson, C. S. and Trifilieff, A. (2006) Effect of adenosine A2A receptor activation in murine models of respiratory disorders. Am. J. Physiol. Lung Cell. Mol. Physiol. 290, L1036-L1043.
152 Gessi, S., Merighi, S., Varani, K., Leung, E., Mac Lennan, S. and Borea, P. A. (2008) The A3 adenosine receptor: an enigmatic player in cell biology. Pharmacol. Ther. 117, 123–140.
153 Gessi, S., Cattabriga, E., Avitabile, A., Gafa', R., Lanza, G., Cavazzini, L., Bianchi, N., Gambari, R., Feo, C., Liboni, A., Gullini, S., Leung, E., Mac-Lennan, S. and Borea, P. A. (2004) Elevated expression of A3 adenosine receptors in human colorectal cancer is reflected in peripheral blood cells. Clin. Cancer Res. 10, 5895–5901.
154 Fishman, P., Bar-Yehuda, S., Madi, L. and Cohn, I. (2002) A3 adenosine receptor as a target for cancer therapy. Anticancer Drugs 13, 437–443.