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Review

Purinergic signaling in the immune system

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ABSTRACT

Extracellular ATP and its metabolite adenosine are increasingly recognized as key mediators of the immune response. Depending on the concentration, ATP may act as an immunostimulant or an immunodepressant, while adenosine is generally acknowledged to be a potent immunosupressor molecule. Signals delivered by extracellular ATP and adenosine are detected and transduced by P2 and P1 receptors, respectively. Virtually all immune cells express P2 and P1 receptors, thus purinergic signaling affects all aspects of immunity and inflammation. This realization has prompted a burst of novel investigations aimed at the design and synthesis of P2- or P1-targeted drugs for the treatment of chronic inflammatory diseases and cancer. In this review we will summarize the most recent developments in this field.

Contents

1. Introduction

A main feature of a homeostatic system is the ability to rapidly sense any signs of distress that might signal the presence of a threat of exogenous or endogenous origin. This is even more important in the case of complex and integrated systems such as living organisms that are characterized by a continuous exchange of information with the external world and at the same time undergo a continuous process of internal self renewal, with the associated generation of new cells as well as elimination of the old ones. Such a homeostatic system must be equipped with a fine network of sensors that constantly monitor the internal environment to detect even the most subtle signs of injury or distress. Accordingly, a homeostatic system should be able to release an array of intracellular messengers capable of signaling cell and tissue damage. In a complex and integrated organism such a signaling network becomes more and more important, thus evolution has selected the easiest and at the same time most efficient signaling devices. The simplest way to signal cell distress is to develop a receptor system that senses in the extracellular space the presence of molecules that are normally sequestered intracellularly. Therefore, it is not a surprise that the most powerful and ubiquitous signal of distress or damage (otherwise known as DAMP, damage-associated molecular pattern) is ATP. Of course, ATP is by no means the only DAMP used by multicellular organisms to signal danger, but it is likely to be the most ancient.

In the healthy organism, ATP is almost exclusively present inside the cells, where it reaches a several millimolar concentration. In the extracellular environment the ATP concentration is negligible, i.e. in the low nanomolar range (Burnstock, 2007). The huge intra/extracellular concentration gradient on the one hand accelerates enormously the speed of release of ATP in response to the opening of plasma membrane ATP-conducing pathways, and on the other increases the signal-to-noise ratio, the background noise being almost nil. Furthermore, ATP is highly water soluble, and thus easily diffusible in the aqueous extracellular environment, and quickly degraded by a battery of powerful nucleotidases (Yegutkin, 2014). Last, but not the least, virtually all eukariotic cells are

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equipped with specific receptors for extracellular ATP, the P2 receptors (Burnstock, 2007). Thus, ATP is an ideal extracellular messenger of cellular distress and P2 receptors ideal sensors of danger. It has been proposed that adenosine, the most important ATP degradation product, might also be an important danger signal (Fredholm, 2007; Sitkovsky and Ohta, 2005), but given its mainly immunosuppressive activity, this nucleoside is more likely to intervene at later stages as an immunoregulatory feedback mediator.

2. Purinergic receptors expressed by immune cells

Four P1 receptor (P1R) subtypes (A1, A2A, A2B and A3), eight P2Y (P2Y1, 2, 4, 6, 11, 12, 13, 14) and seven P2X (P2X1-7) subtypes are known (Burnstock, 2014; Chen et al., 2013; Khakh and North, 2006; North and Surprenant, 2000). Basically all P1 and P2 receptor subtypes are expressed by immune cells, in a cell type- and differentiation-dependent fashion. Adenosine P1 receptors are widely expressed by immune cells of the myeloid and lymphoid lineage. The role of A1 and A3 receptors is not well understood, while there is sound evidence for a crucial role of A2A and A2B receptors in the control of inflammation. P1Rs are differently coupled to mobilization of intracellular Ca^{2+} and cAMP changes, thus activating several stimulatory or inhibitory intracellular pathways. Due to their coupling to cAMP increases, A2A and A2B receptors are mostly immunosuppressive (Antonioli et al., 2013).

P2YRs are also widely expressed in immune cells and have been thoroughly characterized in neutrophilic and eosinophilic granulocytes, monocytes, macrophages, dendritic cells (DCs), T and B lymphocytes. A few P2YR subtypes have also been identified in natural killer (NK) cells. Idzko and co-workers found that P2Y6 receptor expression is strongly upregulated on airway epithelial cells following the induction of allergic airway inflammation (Vieira et al., 2011). P2Y6R was also investigated on DCs, eosinophils, mast cells, monocytes and neutrophils: its activation is associated with pro-inflammatory cytokine release. P2Y12R is the focus of hot interest for its role in coagulation, as it triggers platelet activation and inflammatory mediator release. A direct consequence of these studies has been the development of P2Y12R-targeted antithrombotic therapies (Dorsam and Kunapuli, 2004). Because of their crucial role in angiogenesis, special attention was given to the characterization of P2Y receptors in endothelial cells. There is evidence that P2YRs expressed by $CD31^{+}$ cells, transactivate vascular endothelial growth factor receptor 2 (VEGFR2), thus increasing endothelial cell tubulogenesis in the tumor microenvironment (Rumjahn et al., 2009). Thus, it can be safely concluded that virtually all immune cell types as well as stromal cells express multiple P2YR subtypes (Idzko et al., 2014).

Investigation of P2XRs in immune cells started at about the same time as P2YRs, but developed more slowly since for a long time it was thought that ion channels had a minor relevance in the regulation of immune cell responses. Now this view has substantially changed, a major role for P2XRs in immune regulation has been widely recognized and increasing attention is being paid to their expression and function. Mononuclear phagocytes have been extensively investigated for P2X receptor expression and function, available evidence showing that they express P2X1R, P2X4R and P2X7R (Idzko et al., 2014). P2X5R and P2X6R have been the object of little attention in the immune system, thus not much information is available as to their expression and function. Among mononuclear phagocytes, brain microglia have been extensively investigated for P2X4R and P2X7R expression, both in vitro and in vivo, highlighting the important role played by these receptors in pain and inflammation (Inoue, 2008). Human neutrophils and eosinophils, especially following activation, T and B lymphocytes and NK cells express P2X1R, P2X4R and P2X7R (Idzko et al., 2014). P2X5R expression has been only reported in T lymphocytes (de Rijke et al., 2005). Mast cells express P2X1R, P2X4R and P2X7R and possibly P2X3R (Wareham et al., 2009). Quite interestingly, P2X7R was initially identified and characterized by Gomperts and co-workers in rat mast cells, and named the "ATP^{4-} receptor" (Cockcroft and Gomperts, 1980). However, after those early findings, few additional studies investigated P2XR function in this cell type, despite the obvious relevance in inflammation.

3. Purinergic receptors play a crucial role as stimuli for chemotaxis of inflammatory cells

At the inception of inflammation DAMP signaling is essential to recruit inflammatory cells at the site of tissue damage or pathogen entry. Thus, nucleotides as the primordial DAMPs are anticipated to have chemotactic activity. There is formal proof that ATP is released to micromolar amounts at sites of inflammation or injury (Pellegatti et al., 2008; Weber et al., 2010). Similar demonstration for other nucleotides is lacking, but it is reasonable to believe that their release parallels that of ATP. Once in the extracellular environment, ATP is hydrolyzed by powerful plasma membrane nucleotidases (CD39 and CD73) to generate ADP, AMP and finally adenosine, that is no doubt the most important ATP degradation product (Yegutkin, 2014; Zimmermann, 2000). Nucleotide release generates a concentration gradient sufficient to support cell chemotaxis based on both ATP and adenosine, since directional motility of inflammatory cells requires a close cooperation between these two purines. It has been shown that ATP release and adenosine generation contribute to chemotaxis induced by chemotactic peptides in a space- and time-dependent fashion (Junger, 2011).

In human neutrophils, ATP release during fMet-Leu-Phe-stimulated migration occurs at the leading edge, where it potentiates chemotactic stimulation by acting at the P2Y2R. In this activity, ATP cooperates with extracellular adenosine acting at the A3 receptor in a tightly integrated fashion, where ATP is the basic ingredient of the chemotactic gradient providing the "directional" indications, while adenosine is the stimulus that promotes the actual migration. According to this scheme, P2Y2 receptors are responsible for gradient-sensing and A3 receptors for cell migration (Junger, 2011). For an efficient migration through a purine-based chemotactic gradient, the site of adenosine generation is crucial, and in fact there is evidence that adenosine is generated at the expense of locally-released ATP at the leading edge of chemotacting neutrophils. Other studies support a role of ATP (and ADP) as a true chemotactic signal and not just as a "directional" indicator (Haynes et al., 2006; Honda et al., 2001). On the other hand, some investigators suggest that ATP is not a chemotactic molecule for inflammatory cells, but rather a "conditioning" factor that facilitates the production of other (conventional) chemotactic factors at the inflammatory site (McDonald et al., 2010). According to this view, it is hypothesized that ATP stimulates fibroblasts or resident inflammatory cells (e.g. tissue macrophages) to release mediators responsible for the generation of an inflammatory microenvironment that in turn recruits and activates circulating inflammatory cells. Thus ATP is considered as a signal of cell distress that a) induces the release of chemotactic factors from nearby cells, and b) modulates activation of receptors for chemotactic factors (Kronlage et al., 2010).

The role of nucleotides as true chemotactic factors might be particularly relevant in the context of sterile inflammation, or at sites of extensive apoptotic tissue lesions, as ATP release via pannexins might be involved in the generation of a chemotactic gradient generated by apoptosing cells (Chekeni et al., 2010). Among other nucleotides, UTP has been shown to have a strong chemotactic activity towards neutrophils, mast cells and hematopoietic stem cells (Idzko et al., 2001; Lemoli et al., 2004; Rossi et al., 2007). Receptors involved are the G-protein coupled P2Y receptors, mainly P2Y2, P2Y6, P2Y12 and P2Y13.

The P2Y6R has a special place in inflammation among other P2YRs, including chemotaxis. There is good proof that UDP (and UTP) acting at P2Y6R triggers release of monocyte chemotactic protein 1(MCP1), interleukin (IL)-8 and a number of additional chemokines, thus propagating inflammation and supporting chemotaxis (Eltzschig et al., 2012). Since expression of endothelial adhesion molecules is also enhanced by

P2Y6R stimulation, it is likely that this receptor plays an important role in vascular inflammation and atherosclerosis (Riegel et al., 2011; Stachon et al., 2014). Among the different P2Rs, only P2Y6R was shown to be up-regulated by endothelial cells in response to in vitro stimulation with tumor necrosis factor-α, or in vivo stimulation with lipopolysaccharide (LPS) (Riegel et al., 2011), suggesting that P2Y6R is the main, if not the sole, P2 receptor subtype mediating the early vascular changes supporting diapedesis of inflammatory cells. In this scenario, it is likely that P2YR cooperates with adenosine in the control of vascular inflammation as endothelial cells express CD39 and CD73, especially under hypoxic conditions, and thus adenosine generation is enhanced during inflammation (Antonioli et al., 2013). Genetic deletion of the A2B receptor causes low grade inflammation and increased leukocyte adherence to the vessel wall (Yang et al., 2006). In particular adenosine, acting at A2B receptors, is understood to control expression of intercellular adhesion molecule 1 (ICAM1) and E-selectin, thereby modulating inflammatory cell adhesion to endothelial cells (Antonioli et al., 2013).

Therefore, convincing evidence supports the view that inflammatory cells respond to a graded nucleotide concentration by moving towards the gradient core, where the nucleotide (ATP) concentration is maximal. Once inflammatory cells have reached the core of the inflammatory site, they are exposed to a very high ATP concentration, up to the hundred micromolar level. It is likely that such an elevated ATP level acts as a "stop signal" inhibiting further motility. It is not known if and which P2Rs are involved, but it is probable that P2X7R has a major role to deliver a "stop signal".

Most immune cells have been shown to respond to a nucleotide-based chemotactic gradient, albeit fine details of this migratory activity have been elucidated only for a few, i.e. neutrophils, microglia and DCs. These latter cells are an interesting example of the "logic" that underlies a nucleotide-based chemotactic stimulus. A few years ago, Idzko and co-workers showed that immature versus mature human DCs have a differential migratory ability in response to extracellular nucleotides (Idzko et al., 2002). Immature DCs, i.e. those DCs that have not yet encountered the antigen (Ag), readily respond to stimulation by extracellular nucleotides and progress along the chemotactic gradient Fig. 1. By contrast, mature DCs obtained by in vitro stimulation with LPS to mimic Ag exposure, do not respond to chemotactic stimulation by nucleotides. Lack of response does not depend on failure to express P2Rs but seems to be rather due to uncoupling of P2Rs from the motility apparatus

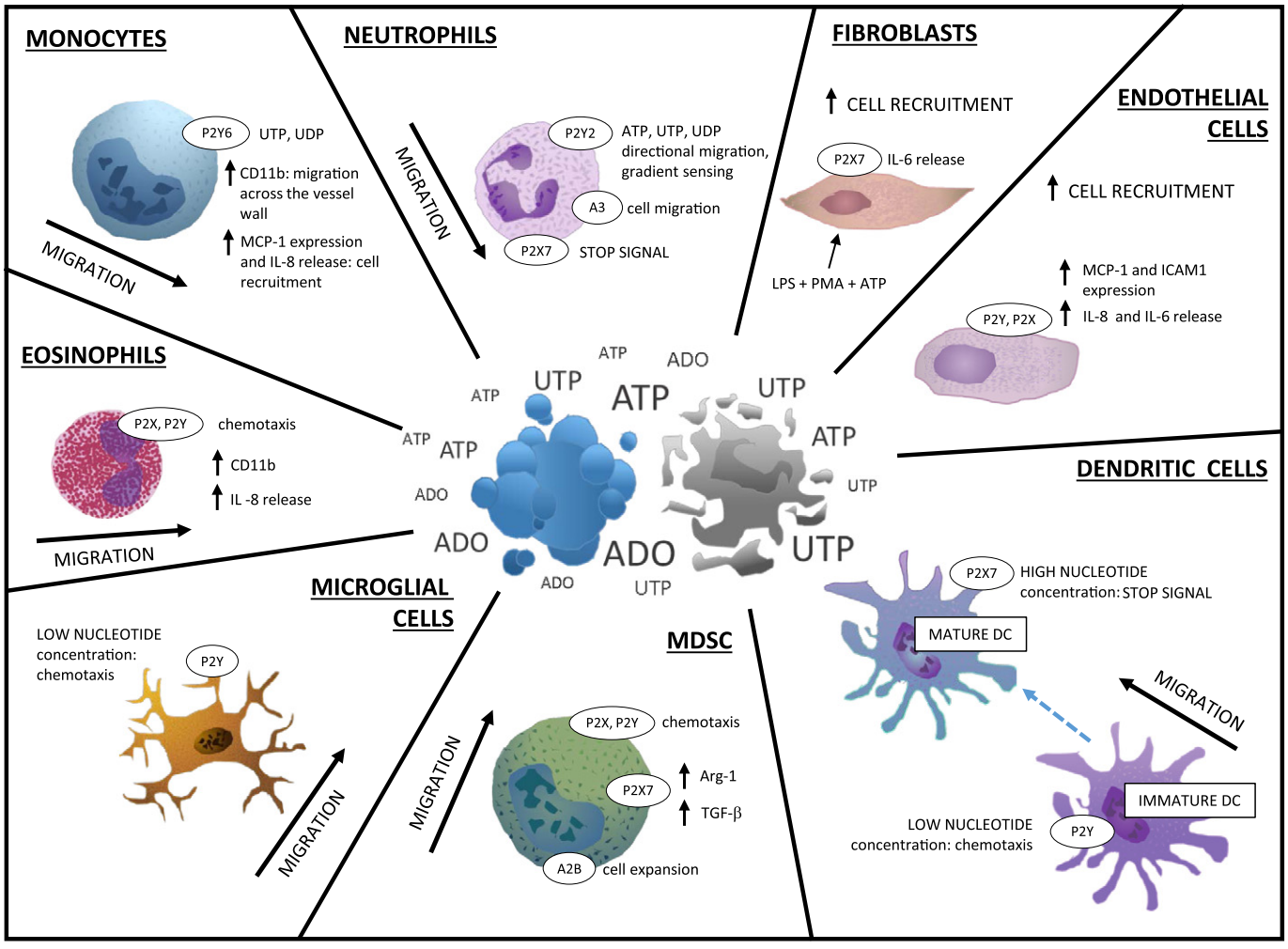

Fig. 1. Schematic overview of the chemotactic effect of ATP and adenosine on inflammatory cells. Purinergic signaling plays a pivotal role in the chemotaxis of multiple cell types. Extracellular nucleotides attract inflammatory cells to the inflammation core either by driving directional cell motility or by inducing the release of chemotactic factors from stromal or resident inflammatory cells. Among other nucleotides, UTP has been shown to have a strong chemotactic activity towards neutrophils, monocytes, DCs and myeloid-derived suppressor cells (MDSC). In human monocytes and eosinophils UTP and UDP trigger cell migration, CD11b expression and IL-8 release, thus propagating inflammation and cell recruitment. Similarly, fibroblasts and endothelial cells release monocyte chemotactic protein 1(MCP1) and IL-6. Low nucleotide concentrations, acting at P2Y receptors, are a strong stimulus for chemotaxis, while on the contrary, the very high ATP level at the inflammation core acts as a "stop signal", inhibiting any further cell motility, probably in a P2X7R-dependend manner. In some cases, e.g. human neutrophils, chemotaxis is the result of a close cooperation between ATP and extracellular adenosine. The first provides the "directional" indications and the second, acting at the A3 receptor, promotes the actual cell migration.

(Idzko et al., 2002). This finding highlights the crucial role of extracellular nucleotides in the activation of the initial steps of inflammation and immunity.

In fact, the role of extracellular ATP as a DAMP is exactly that to provide: a) a "second" (permissive?) signal that enables DC activation once they have captured the Ag, and b) generate a chemotactic gradient that promotes their migration to the lymph nodes, where they meet Ag-specific T lymphocytes. Immature DCs during this itinerary across an increased ATP gradient differentiate to become mature DCs, with an enhanced ability for Ag-presentation and T lymphocyte stimulation.

There is no other demonstration of a similar selective migratory behavior depending on the maturation/differentiation state of an immune cell, but it is likely that most phagocyte cells with Ag-presenting ability react to a nucleotide-based chemotactic gradient in this same way.

4. Extracellular purines and Ag recognition and destruction

As expected from a bona fide DAMP, ATP modulates DC functions in multiple fashions. DCs are known to skew $CD4^+$ T lymphocyte differentiation towards a Th1, Th2, Th17 or Treg phenotype (Walsh and Mills, 2013). Several factors are understood to affect this process, among which adenosine and extracellular ATP seem to play an important role (la Sala et al., 2001; Panther et al., 2003). The inflammatory microenvironment is rich in extracellular ATP that, in conjunction with other pro-inflammatory factors, skews DC maturation towards a Th2 phenotype (la Sala et al., 2002). In addition, IL-6 stimulation of Treg cells promotes ATP autocrine/paracrine secretion and their conversion to Th17 cells (Schenk et al., 2011). There is evidence that extracellular ATP signaling is also involved in shaping T lymphocyte differentiation versus a γ/δ rather than an α/β phenotype (Frascoli et al., 2012). Adenosine accumulating at inflammatory sites, besides modulating DC differentiation, also affects macrophage and lymphocyte functions as well as release of VEGF (Antonioli et al., 2013). Stimulation of A2B receptors drives an anomalous DC differentiation to a "tumor-promoting" phenotype characterized by decreased CD1a expression, reduced CXCL10 production, lower tumor antigen cross presentation and increased VEGF secretion (Fig. 2) (Antonioli et al., 2013). In addition, adenosine acting at A2B receptors promotes the expansion of cells involved in immunosuppression such as myeloid-derived suppressor cells (MDSCs) (Antonioli et al., 2013).

The effect of extracellular purines on immunosuppressive pathways is an expanding field of interest. Recent findings by Bianchi et al. revealed that extracellular ATP might also have an important role in immunosuppression since mouse MDSCs overexpress the P2X7R that in this cell type is coupled to release of immunosuppressive factors, such as arginase-1 and TGFβ (Bianchi et al., 2014). Given the extremely high concentration of extracellular adenosine and ATP within the tumor microenvironment, it is highly probable that purinergic signaling has a crucial effect on tumor progression by directly stimulating tumor growth and by suppressing the anti-tumor immune response. Incidentally, the investigation of MDSC

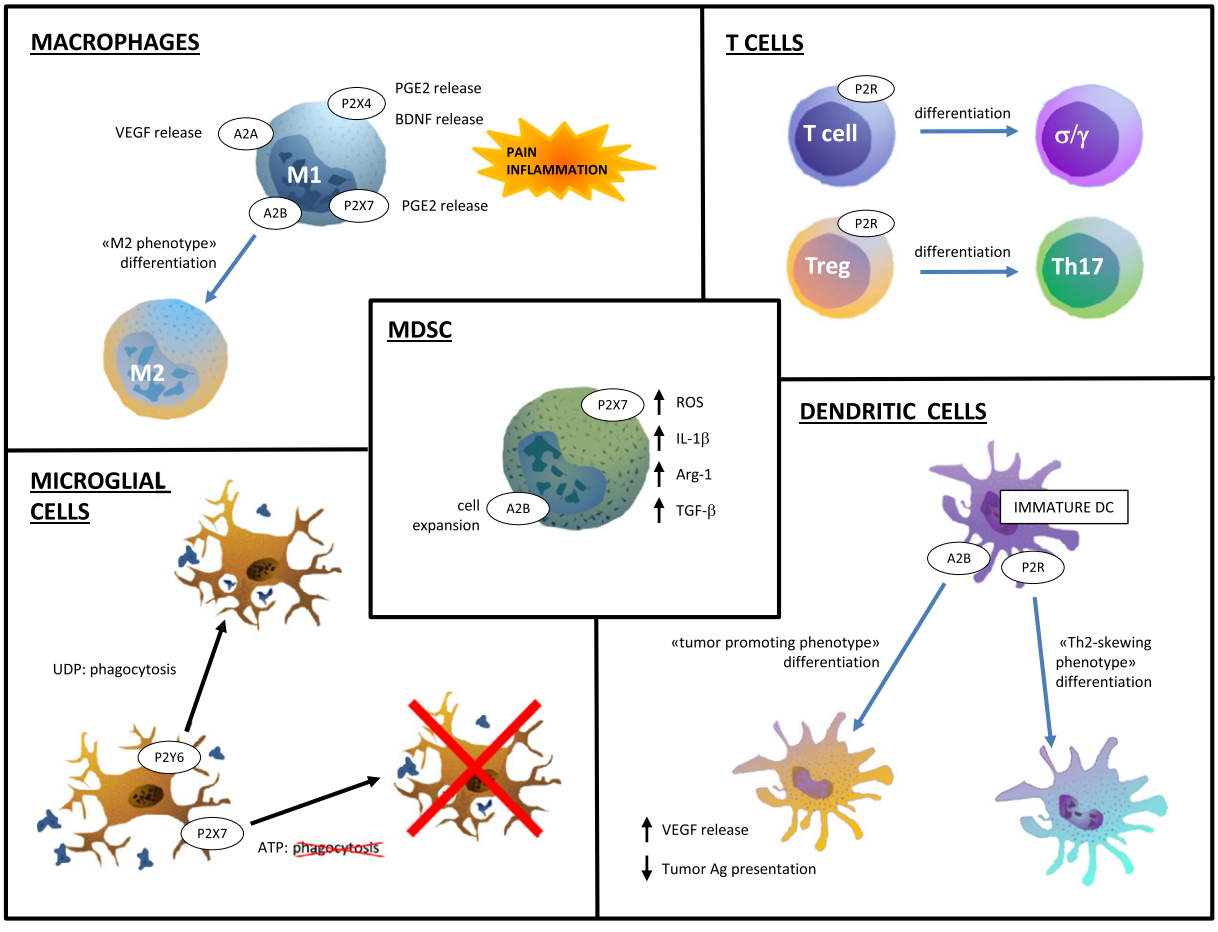

Fig. 2. Pathophysiology of purinergic signaling. Extracellular nucleotides exert a wide range of effects on immune cells, with important implications in many diseases. Growing evidence support the role of P2X7 receptor in pain and fever. It has been recently observed that activated human and murine macrophages release PGE2 and brain-derived neurotrophic factor (BDNF) in response to extracellular ATP stimulation. There is also evidence that extracellular ATP drives T lymphocytes differentiation to a γ/δ phenotype and DCs to a Th2-skewing phenotype. Moreover, the autocrine/paracrine ATP secretion by Tregs, in response to IL-6, induces their conversion to Th17 cells. In microglial cells extracellular UDP promotes phagocytosis in a P2Y6R dependent manner, while extracellular ATP acts as an inhibitor, likely via P2X7R. Purinergic signaling might also be implicated in tumor immune escape. In MDSCs, P2X7R activation induces Arg-1, TGF-β and ROS release. Extracellular adenosine, acting at A2B receptor, promotes MDSC proliferation and macrophages differentiation into M2 phenotype. A2B receptor is also involved in reduced tumor Ag presentation and VEGF release by DCs.

P2X7R is provides the additional surprising demonstration of the peculiar plasticity of this receptor that, well known for mediating a strong cytotoxic response, in these cells appears to be almost entirely uncoupled from cytotoxicity. Whether this is an adaptation of MDSCs to a high ATP environment is an interesting pathophysiological question.

The nucleotide-rich inflammatory milieu strongly modulates additional key responses of immune cells such as phagocytosis, phagosome maturation and secretion of cytotoxic factors, in a cell type- and nucleotide-dependent fashion. UDP acting at microglia P2Y6R is a potent pro-phagocytic stimulus in the central nervous system where it might function as a signal of neuronal distress and injury (Koizumi et al., 2007). In another experimental setting P2Y6R blockade was sufficient to prevent neuronal loss induced by inflammatory stimuli in glial/neuronal cultures (Neher et al., 2014). Whether UDP and P2Y6R play a similar role in other immune cells is not known. At variance with UDP, ATP has an inhibitory effect on phagocytosis, likely mediated via the P2X7R. It has to be stressed however that little effort has been made to identify the molecular mechanism responsible for this inhibitory effect, and it is not even clear whether it is physiologically relevant, since it might simply be the consequence of the well known P2X7-dependent cytotoxic activity. Full understanding of the physiological meaning of P2X7R-dependent inhibition of phagocytosis is further complicated by the well documented, and apparently paradoxical, effect of P2X7R stimulation on phagosome maturation.

It is long known that P2X7R stimulation accelerates phagosome–lysosome fusion, and that this process is responsible for the accelerated clearance of parasites that normally survive phagocytosis such as *Mycobacterium tuberculosis* or *Chlamydia psittaci* (Coutinho-Silva et al., 2003; Lammas et al., 1997). The mechanism whereby P2X7R-stimulation triggers phagosome–lysosome function is far from clear. It was originally thought to be a side effect of apoptosis (Molloy et al., 1994), but contrary to this view, there is evidence that the effect on intracellular vesicle fusion can be dissociated from the cytotoxic effect (Fairbairn et al., 2001). Hints that P2X7R, and other P2XRs such as P2X4R, might have a so far unsuspected direct role in supporting trafficking and fusion of intracellular membranes comes from the long standing observation that P2X7R stimulation triggers recycling and fusion to the plasma membrane of intracellular endosome-like vesicles (Morelli et al., 2003). Furthermore, intracellular vesicle–vesicle fusion which leads to formation of large macro-vesicles is also enhanced. Murrel-Lagnado and co-workers have shown that P2X4R is enriched in the lysosomal membranes where it is activated by luminal ATP in a pH-dependent fashion (Huang et al., 2014). If we put these findings together with earlier studies by North and co-workers reporting the presence in *Dictyostelium discoideum* of a P2X-type receptor involved in osmoregulation, it seems clear that the P2XR family as a whole has an important function in the regulation of intracellular vesicle physiology (Fountain et al., 2007). In general terms, we are tempted to propose that P2XRs have an important and widespread role as modulators of membrane fusion, whether at the plasma membrane or organelle membrane level. As early as 1995 we showed that increased expression of P2X7R by activated macrophages was associated to differentiation of these cells to multinucleated giant cells, and therefore to their ability to fuse with each other and generate syncytia (Falzoni et al., 1995). In several follow-up studies we showed that monocyte cell monolayers concentrate P2X7R at sites of cell-to-cell interaction, and that blockade of this receptor fully prevents multinucleated cell formation (Chiozzi et al., 1997; Falzoni et al., 2000; Lemaire et al., 2006, 2011). Regrettably, however, the relevance of these findings to human pathology has not been thoroughly investigated so far.

While the role of P2XRs in membrane fusion is not fully understood, convincing evidence supports a major function of P2X7R in intracellular pathogen killing (Miller et al., 2011). In fact, there is little doubt that P2X7R activation strongly potentiates intracellular parasite killing and therefore is of potential importance in host defense against pathogens. By speeding up phagosome–lysosome fusion P2X7R increases exposure of intraphagosomal microorganisms to lysosomal degrading enzymes and to reactive oxygen species (ROS) elaborated by phagocyte NADPH oxidase, or by the mitochondrial respiratory chain. Albeit neither the specific P2R subtype responsible for phagocyte cell degranulation and NADPH oxidase activation, nor the causative mechanism have ever been investigated in detail, scattered evidence suggest that both P2YRs and the P2X7R are involved.

Over-activation of P2X7R is a potent uncoupling stimulus for the mitochondrial respiratory chain, and by consequence a strong stimulant of mitochondrial ROS formation. This process has become a focus of hot interest by inflammation students since there is growing evidence that ROS generated by the mitochondria are a pivotal factor in NLRP3 inflammasome activation (Zhou et al., 2011). Thus, P2X7R is not only the main (sole?) pathway causing the cytoplasmic K^+ drop univocally thought to be the common mechanism shared by all NLRP3 inflammasome-activating extracellular agents (Munoz-Planillo et al., 2013), but also the stimulus for the generation of ROS, an additional and powerful intracellular NLRP3 activator. To our knowledge, there are no other plasma membrane receptors of immune or inflammatory cells with similar functions. Given the undisputed role of the NLRP3 inflammasome in the maturation and release of IL-1β, the prototypical pro-inflammatory cytokine, there is no doubt that the P2X7R-mitochondria axis is crucial in the initial phases of inflammation.

P2X7R, and to a lesser extent P2X4, participate in the release of additional inflammatory mediators. In mice, the P2X4R is an effective stimulus for prostaglandin E2 (PGE2) release, but in humans P2X7R appears to be much more potent (Barbera-Cremades et al., 2012; Ulmann et al., 2010). Pelegrin and co-workers have shown that relatively high (millimolar) ATP doses trigger PGE2 release from LPS-primed human and murine macrophages (Barbera-Cremades et al., 2012). PGE2 release is blocked by selective P2X7 blockers and totally abrogated in P2X7-KO mice. Interestingly, deletion of P2X4 did not affect PGE2 release stimulated by high extracellular ATP. On the other hand, low (micromolar) ATP concentrations, subthreshold for P2X7 stimulation, were able to elicit modest PGE2 release, as described by Rassendren and co-workers (Ulmann et al., 2010). P2X4R may also contribute to inflammation by sustaining the release of brain-derived neurotrophic factor (BDNF) (Beggs et al., 2012; Coull et al., 2005). BDNF is well known to be able to cause aberrant nociception, and therefore neuropathic pain (allodynia). However, BDNF is also implicated in neuroinflammation and in neuropsychiatric disorders, thus P2X4R activation might also indirectly contribute to these pathologies by sustaining BDNF release (Skaper et al., 2010).

5. Conclusion

Although purinergic signaling was initially thought to be an essential and physiologically relevant signaling pathway exclusively in the nervous system, it is increasingly evident that it also plays an essential, possibly even more important, role in the immune and inflammatory systems. A literature (December 2014) search linking "purinergic receptors" to inflammation or immunity yielded over 1650 hits, while a similar search linking "purinergic receptors" to central or peripheral nervous system yielded about 6000 hits. However, while the number of reports on the nervous system plateaued in 2001–2003, reports on inflammation and immunity are still steadily increasing, thus highlighting the topicality of purinergic research in immunology. Given the key role of inflammation and immunity in basically all human diseases, it is quite obvious that a thorough investigation of the biology and pharmacology of purinergic signaling will open novel avenues for therapy.

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