Review

Purinergic signalling in inflammation of the central nervous system

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Inflammation is the most fundamental body reaction to noxious stimuli. No vascularized tissue, organ or apparatus is free from this response. Several mediators of inflammation, originating from outside (exogenous) or inside (endogenous) the body, are known. Among the endogenous factors, extracellular nucleotides and nucleosides are attracting interest for their ubiquity and striking ability to modulate diverse immune responses. Until recently, it was doubted that the central nervous system (CNS), reportedly an 'immunoprivileged organ', could be the site of immune reactions. Nowadays, it is acknowledged that inflammation and immunity have a key role in a vast range of CNS diseases. Likewise, it is clear that purinergic signalling profoundly affects neuroinflammation. Here, we provide a brief update of the state of the art in this expanding field.

Introduction

Inflammation is a complex homeostatic mechanism devised to protect the integrity of the organism against endogenous or exogenous noxious agents. It is usually considered an aspect of innate immunity or, according to some, innate immunity tout court [1,2]. Inflammation has been known to physicians for over two thousand years and its basic manifestations (cardinal signs) *calor* (warmth), *dolor* (pain), *tumor* (swelling) and *rubor* (redness) were clearly identified by Aulus Cornelius Celsus in the early years of the Christian age. *Calor*, *dolor*, *tumor* and *rubor* describe and summarize all the crucial events occurring in a tissue during the inflammatory response.

Players of inflammation are cells and soluble factors. The family of inflammatory cells has now grown to include cell types as different as fibroblasts, endothelial cells and adipocytes besides the typical circulating and resident leukocytes and tissue mast cell [2]. However, their increase in number is negligible if compared to the exponential growth of novel soluble inflammatory mediators, now numbering in hundreds. Among these, extracellular adenine and uracil nucleotides (e.g. ATP and UTP) and nucleosides (e.g. adenosine) acting at P2 or P1 purinergic receptors are relative new-comers in the field [3,4]. In addition, enzymes that degrade extracellular nucleotides, such as ectoATP/ADPases (CD39) and ectoAMPase (CD73), also have a profound immunomodulatory activity [5]. Here, as a follow up to the first [6] of a series of articles dealing with purinergic signalling in the central nervous system (CNS), we summarize data highlighting a pivotal role for this system in alerting and tuning immune and inflammatory reactions to aversive influences in the CNS. We also highlight recent developments that implicate purinergic signalling in acute and chronic neurodegenerative diseases, hoping that a better understanding of the role of extracellular purines and pyrimidines in neuroinflammation will lead to the development of novel therapies.

A brief history of purinergic signalling in inflammation

Many metabolites and the enzymes responsible for their generation (e.g. nitric oxide and the inducible nitric oxide synthase, arginine and arginase, tryptophan metabolites and indoleamine deoxygenase, lipoxins and lipoxigenase and, more recently, nucleotides, adenosine and their metabolizing enzymes, i.e. CD39 and CD73) are now regarded as true inflammatory mediators. Players in purinergic signalling are extracellular nucleotides, adenosine, CD39 and CD73. Here, we only focus on the role of nucleotides in inflammation; for adenosine, CD39 and CD73 the reader is referred to recent exhaustive reviews [7–10].

Indications of an involvement of extracellular ATP in systemic inflammation date back to the 1970 s when Dahlquist and Diamant [11] reported its strong histamine-releasing action from rat mast cells. Cockcroft and Gomperts [12] later postulated expression by mast cells of a specific ATP receptor, which was later identified as the $P2X_7$ receptor. These early observations were followed by scattered reports of the ability of this nucleotide to produce responses in lymphocytes, monocytes and polymorphonuclear granulocytes (for review, see Ref. [13]). However, the notion that extracellular ATP might participate in inflammation gained acceptance very slowly, at least until P2 receptors were cloned and their expression by inflammatory cells was fully characterized. The turning point was the discovery of the strong linkage of one of the P2 receptors (the ATP-gated $P2X_7$ receptor channel) to maturation and secretion of the key cytokine interleukin (IL)-1β [14–16] and the *in vivo* demonstration that ATP is present in high levels in the extracellular space during inflammation [17,18]. To date, a thorough characterization has been made of P2 receptor expression, not only of the seven subtypes of ligand-gated $P2X_1$–$P2X_7$ receptor but also of the $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ G-protein-coupled

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metabotropic-receptor subtypes [19], in most inflammatory cells, even though changes in P2 receptor expression in diseased states have been so far only superficially explored. P2 receptors are involved in inflammation at different levels and with different roles. Overall, $P2X_7$ has the most clear-cut role, but this might be simply owing to the fact that a potent blocker (oxidized ATP) and a clear physiological response (maturation and release of IL-1β) for this receptor were very early identified [20,21]. Among other P2 receptors, $P2Y_2$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$ and $P2X_4$, are those for which a closer link with inflammation has been unveiled. The $P2Y_2$ receptor has been recently shown to have a crucial role in the orientation of neutrophils in a chemotactic gradient [22], whereas $P2Y_6$ has been implicated in intestinal inflammation and in release of CXCL8 (also known as IL-8), IL-6 and macrophage inflammatory protein-2 [23,24]. By contrast, $P2Y_{11}$ plays an important part at the interface between native and adoptive immunity because stimulation of this receptor by extracellular ATP induces a distorted maturation of human dendritic cells (DCs) that, in turn, skews $CD4^+$ lymphocytes towards a Th2 phenotype [25–27]. Finally, the recent discovery that CD39 is a marker of immunosuppressive $Foxp3^+$ Treg cells further underscores the action of ATP as an immunomodulator [28,29]. The part played by $P2Y_6$, $P2Y_{12}$, $P2X_4$ and $P2X_7$ will be described in the following section. As summarized elsewhere [6,30], both P2X and P2Y receptors and CD39 and CD73 are expressed on all types of nervous system cells; these are neurons, oligodendrocytes and the two types of glial cells involved in inflammatory reactions (microglia and astrocytes). However, the concept that purinergic signalling is crucial in neuroinflammation is rather recent and still evolving acquisition.

Microglial cells, the resident CNS immunocytes, carry multiple nucleotide receptors involved in inflammation

It was widely thought that the CNS is an immunoprivileged organ thanks to the blood–brain barrier (BBB). However, there is now ample evidence that this assumption in untenable. The CNS hosts resident immune cells (microglia and probably other 'non canonical' immune cells such as the astrocytes) that react to pathogens or damage, migrate to the site of injury, phagocytose invading microorganisms or cell debris and elaborate and secrete cytokines, chemokines and other inflammatory mediators. Furthermore, T and B lymphocytes invade the CNS during distinct neuropathologies (e.g. multiple sclerosis, Alzheimer's disease or adrenoleukodystrophy) and activate resident or infiltrating inflammatory cells, thus causing CNS injury [31].

Participation of microglia, the resident immune cells of the CNS, to almost all neurological diseases is nowadays an established fact [31,32]. Thus, it is a lucky coincidence that experiments performed in this very cell type in the mid nineties laid the foundation of our understanding of purinergic signalling in inflammation [15,33–36]. Expression of several P2 and adenosine receptor subtypes and modulation by extracellular nucleotides and nucleosides of multiple microglia *in vitro* and *in vivo* responses, such as proliferation, process motility, migration, phagocytosis, cytokine and chemokine release, makes this cell type a paradigm for purinergic studies in inflammation [31].

Microglia was the first cell type in which coupling of the $P2X_7$ receptor to IL-1β release was formally shown [15]. Indeed, much of our current understanding of the close link between purinergic receptors and neuroinflammation originates from the serendipitous finding that the $P2X_7$ receptor is highly expressed and strictly coupled to maturation and release of this cytokine in microglia. Over the years, $P2X_7$-mediated microglia responses were extended to include cytokine and chemokine secretion in addition to the release of activated oxygen species and proliferation [31,37,38]. Other receptor subtypes such as $P2X_4$, $P2Y_6$ and $P2Y_{12}$ are now strongly implicated in microglia-mediated neuroinflammation. After nerve injury, expression of the $P2X_4$ receptor increases strikingly in ipsilateral hyperactive microglia (but not in neurons or astrocytes) and mediates tactile allodynia [39]. Intraspinal administration of a $P2X_4$ receptor antisense oligodeoxynucleotide suppresses tactile allodynia in this model. Conversely, intraspinal transplantation of microglia expressing activated $P2X_4$ produces tactile allodynia in unchallenged rats. The $P2Y_6$ receptor is upregulated upon brain damage and triggers phagocytosis in response to exogenously added or endogenously released UDP [40]. $P2Y_{12}$ receptor expression is dramatically increased in microglial cells ipsilateral to peripheral nerve injury in the spinal cord [41,42] but down-modulated in activated microglia in the brain [43].

Inoue and Kohsaka [44,45] have investigated in depth the mechanism responsible for ATP-mediated chemotaxis in microglia identifying $P2Y_{12}$ and $P2X_4$ as the receptors involved. Generation of an ATP concentration gradient is a smart mechanism to achieve a graded activation of microglia. Low ATP concentrations activate almost exclusively chemotaxis to recruit cells at the injured or inflamed site. Then, when ATP concentration increases, additional effector functions such as phagocytosis and cytokine secretion are also triggered. In $P2Y_{12}$-receptor-null mice undergoing focal laser cortical ablation, the chemotactic response of resting microglia was markedly impaired in the first 40 min observation period compared with wild-type animals [43]. However, when microglia from mutant mice were examined 2 h after injury, the degree of chemotaxis approached that observed in wild-type controls. Moreover, 24 h after injury, $P2Y_{12}$ receptor expression on microglia was barely observable. Loss of $P2Y_{12}$ expression accompanied microglial transformation from highly ramified to amoeboid state. These observations indicate that $P2Y_{12}$ receptors are involved in the early, rather than late, responses of microglia to injury [43].

Thus, during brain damage or inflammation, nucleotides can serve as 'find-me' and 'eat-me' signals [46]. Injured or necrotic cells release large amounts of ATP or UTP, which are then converted to ADP and UDP by ectonucleotidases. By selectively activating $P2Y_{12}$, ATP and ADP recruit microglia and promote chemotaxis, whereas UDP facilitates phagocytosis of dying cells via $P2Y_6$ receptors (Figure 1).

Multiple P2X and P2Y receptors cooperate to induce inflammatory reactive astrogliosis

Multiple P2X and P2Y receptors are also expressed by astrocytes in both the central and peripheral nervous