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The role of danger signals and ectonucleotidases in acute graft-versus-host disease

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ABSTRACT

Allogeneic hematopoietic cell transplantation (allo-HCT) represents the only curative treatment approach for many patients with benign or malignant diseases of the hematopoietic system. However, post-transplant morbidity and mortality are significantly increased by the development of acute graft-versus-host disease (GvHD). While alloreactive T cells act as the main cellular mediator of the GvH reaction, recent evidence suggests a critical role of the innate immune system in the early stages of GvHD initiation. Danger-associated molecular patterns released from the intracellular space as well as from the extracellular matrix activate antigen-presenting cells and set pro-inflammatory pathways in motion. This review gives an overview about danger signals representing therapeutic targets with a clinical perspective with a particular focus on extracellular nucleotides and ectonucleotidases.

1. Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) is an increasingly used often curative treatment approach for patients with benign and malignant diseases of the hematopoietic system. However, even with complete major histocompatibility complex (MHC)-match between donor and recipient, about 40–60% of allo-HCT recipients develop acute graft-versus-host disease (GvHD) [1], a potentially lethal immunologic complication mediated by activation of donor T cells which mount an attack against host tissues which they recognize as foreign. Besides T cells, various other immune cell populations are involved in the pathogenesis of GvHD, including dendritic cells, neutrophils, inflammatory monocytes, B cells and also non-hematopoietic cells, e.g. endothelial cells and enterocytes participate in the disease development [2]. The current state of knowledge distinguishes three stages in

Abbreviations: A2A-AR, A2A-adenosine receptor; ADP, adenosine diphosphate; Allo-HCT, allogeneic hematopoietic cell transplantation; AMP, adenosine monophosphate; APC, antigen-presenting cell; AR, adenosine receptor; ATP, adenosine triphosphate; ASC, apoptosis-associated speck-like protein containing a carboxy-terminal CARD; cAMP, cyclic adenosine monophosphate; CARD, caspase recruiting domain; CCL, chemokine ligand; CREB, cAMP response element-binding protein; CD, cluster of differentiation; DAMP, danger-associated molecular pattern; DC, dendritic cell; ERK, extracellular-signal regulated kinase; GvHD, graft-versus-host disease; GvL, graft-versus-leukemia effect; HIF, hypoxia-inducible factor; HLA, human leukocyte antigen; HMGB1, high-mobility group box protein 1; Hsp, heat shock protein; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; LRR, leucine-rich repeat; MAP, mitogen activated protein; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; MSC, mesenchymal stromal/stem cell; NF-κB, nuclear factor 'kappa-light-chain-enhancer' of activated B-cells; NOD, nucleotide oligomerization domain; NLR, NOD-like receptor; NLRP3, NOD-like receptor family, pyrin domain containing 3; NPP, nucleotide pyrophosphatase/phosphodiesterase; NTP, nucleotide triphosphate; NTPDase, ecto-nucleotide triphosphate diphosphohydrolase; PAMP, pathogen-associated molecular pattern; PLC, phospholipase C; PRR, pattern-recognition receptor; RAGE, receptor for advanced glycation end products; Reg3α, regenerating islet-derived protein 3-alpha; RNA, ribonucleic acid; ROS, reactive oxygen species; ST2, suppression of tumorigenicity 2; TCR, T cell receptor; Th1, T helper cells 1; Th2, T helper cells 2; Th17, T helper cells 17; TGF-β, tumor growth factor β; TLR, toll-like receptor; TNF, tumor necrosis factor; Treg, regulatory T cell; UDP, uridine diphosphate; UTP, uridine triphosphate; UVB, ultraviolet B light.

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acute GvHD pathogenesis [1,2]. In the early phase, tissue damage by the conditioning treatment leads to release of bacterial products (pathogen-associated molecular patterns, PAMPs), as well as danger-associated molecular patterns (DAMPs), mostly intracellular components which act as pro-inflammatory agents once they are released in the extracellular space. PAMPs and DAMPs activate the innate immune system by binding to cell surface receptors of different families which in turn leads to a pro-inflammatory microenvironment and promotes allogeneic T cell priming. In this way, PAMPs and DAMPs play an important role in the development and persistence of GvHD. After priming, T cells migrate to the acute GvHD target organs, mainly skin, liver and the gastrointestinal tract and induce cell damage by necrosis and apoptosis. Simultaneously, hematopoietic and non-hematopoietic cells release pro-inflammatory cytokines, e.g. TNFα, IFN-γ, IL-1β leading to a "cytokine storm" which is a hallmark of acute GvHD.

This review summarizes the role of various DAMPs in the pathogenesis of GvHD and focuses in particular on purinergic receptor signaling and metabolism via enzymes known as ectonucleotidases.

An overview about the discussed pathways is given in Table 1.

2. Danger signals and their receptors

Activation of the immune system occurs naturally after contact with pathogens or pathogen products such as lipopolysaccharide (LPS) or bacterial nucleic acids. But also during sterile inflammation as it occurs in autoimmune diseases or wound healing, there are messenger molecules which activate the immune system. These endogenous signaling molecules are known as DAMPs, danger signals or alarmins [3,4]. They can be derived either from damaged cells where they are found in high concentrations within the cytoplasm or the nucleus or also from the extracellular matrix [5]. Danger signals, derived from the intracellular or intranuclear compartment include small molecules such adenosine triphosphate (ATP) [6,7], uric acid [8], heat shock proteins (HSP) [9], the high-mobility group box protein 1 (HMGB1) [10,11] or S100 protein family members [12,13]. Components of the extracellular matrix can also be sequestrated and serve as DAMPs, e.g. biglycan [14,15], heparan sulfate [16], fibronectin [17] or fibrinogen [18]. Danger signals activate the innate immune system by interaction with pattern-recognition receptors (PRR) including toll-like receptors (TLR), NOD-like receptors (NLR) as well as purinergic and interleukin receptors [19].

TLR are the best characterized family of PRR which recognize many bacterial-, fungal- or viral-derived PAMPs as well as various DAMPs [20–22]. The TLR family comprises 10 members in humans and 13 in the mouse, which are located partly on the cell surface and partly in the cytosol. Recognized ligands include LPS, flagellin as well as viral and bacterial RNA. Upon ligand binding, TLR recruit adaptor proteins, e.g. MyD88 and TRIF which in turn activate pro-inflammatory pathways such as the NF-κB and the MAPK-pathway and lead to the transcription of various cytokines [22].

Nucleotide oligomerization domain (NOD)-like receptors (NLR) are intracellular cytosolic receptors which are involved similarly to TLR primarily in pathogen recognition. This receptor family consists of at least 23 members in humans and at least 34 members in the mouse. Three domains are characteristic for most NLR: first, a centrally located NOD (also addressed as NACHT cassette) which regulates the self-oligomerization in an ATP-dependent manner; second, C-terminal leucine-rich-repeats (LRRs) which bind the ligands; third, a variable N-terminal domain which might consist of caspase recruiting domain (CARD), pyrin domain, acidic domain or baculovirus inhibitor repeats and mediates protein-protein interactions with downstream molecules [23]. NLR signaling through Nod1 and Nod2 converges with TLR signaling to regulate the activation of the NF-κB and the MAPK pathway generating a host response mostly to bacterial pathogens but also to endogenous DAMPs.

Purinergic receptors represent another class which can activate antigen-presenting cells upon DAMP binding. These receptors bind extracellular nucleotides such as adenosine triphosphate (ATP), adenosine diphosphate (ADP) or uridine triphosphate (UTP) and nucleosides, e.g. adenosine. There are 2 major families of purinergic receptors, the P1 and the P2 receptor families. The P1 receptor family comprises four members (A1, A2A, A2B and A3). The P2 family can be divided into two subfamilies: the P2X receptors, which are ligand-gated ion channels; and the P2Y receptors which are G-protein-coupled. P1 receptors recognize mostly adenosine and are also G-protein coupled. While the A2A and the A2B-receptor interact with a G_s protein and suppress cellular responses by increasing cAMP levels, the A1 and the A3 receptor activate a G_i and a G_q protein respectively and promote cell activation. P2X receptors regulate cellular responses by changing the ion flux rates, whereas P2Y receptors mediate their effects via cAMP generation and phospholipase C (PLC) signaling [24].

In the following we will discuss the role of various danger signals for the pathogenesis of acute GvHD in pre-clinical and clinical models.

3. ATP and activation of the inflammasome

ATP is found in high concentrations within the cell and the mitochondria as the universal energy source of the cell, whereas the extracellular amounts are in the low nanomolar range [25]. Upon cell death, ATP is released from damaged cells and the concentration in the extracellular space rises rapidly. ATP is sensed by the P2 receptor family, which is expressed by a variety of hematopoietic and non-hematopoietic cell types. Extracellular levels of ATP are regulated via ectonucleotidases, cell membrane enzymes which hydrolyze nucleotides to the respective nucleosides. Most prominently, ATP is metabolized by CD39 (NTPDase-1) to ADP and AMP, and ecto-5′-nucleotidase (CD73) generates adenosine. Adenosine itself serves as a potent anti-inflammatory ligand of the P1 receptor family.

Accumulation of extracellular ATP is known to function as a potent DAMP and can be sensed by a variety of cell populations which are engaged in the GvHD development. For instance, increased levels of extracellular ATP induce dendritic cell (DC) maturation with upregulation of costimulatory molecules, production of IL-12 and improved capacity to stimulate allogeneic T cells [7]. Besides, TNFα and ATP were reported to act synergistically in DC activation [7]. Stimulation of the P2X7 receptor in particular leads to release of IL-1β-loaded vesicles from mature DC which additionally contain caspase-1 and caspase-3 as well as cathepsin D [26]. However, prolonged ATP exposure can also induce cell death [27]. Additionally, ATP stimulation promotes a chemotactic response in mature, but not in immature DC [28,29]. Nevertheless, the formation of ATP gradients, as it occurs in sites of injury and acute inflammation, might inhibit DC migration leading to a temporary restrain in areas with high antigen density [30]. With regard to functional aspects, ATP was shown to promote airway inflammation by attracting lung myeloid DC that drive Th2 cell polarization and responses in mediastinal lymph nodes [31].

Neutrophil granulocytes represent another innate immune cell population which plays a pivotal role in GvHD pathogenesis [32] and might be recruited by extracellular nucleotides. P2Y6 receptor signaling mediates IL-8 production in human neutrophils and augments chemotaxis of immature DC, monocytes and T cells [33]. Additionally, the P2Y11 receptor contributes to the chemotaxis of