Activation of Innate Immunity in Graft-versus-Host Disease: Implications for Novel Targets?

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Keywords
Graft-versus-host disease · Danger-associated molecular patterns · Innate immunity · Adenosine triphosphate · Uric acid · Purinergic receptors · Pattern recognition receptors · Syk · JAK1/2 · RelB

Summary
Acute graft-versus-host disease (GvHD) is mediated by alloreactive donor-derived T cells with a suitable T cell receptor recognizing recipient major histocompatibility complex or minor histocompatibility antigens. However, the process of T cell activation and tissue injury sensing is also dependent on innate immune cells and non-hematopoietic cells. Different cell types of the innate immune system have the ability to sense danger-associated and pathogen-associated molecular patterns via pattern recognition receptors which can be transmembrane Toll-like receptors or cytoplasmic nucleotide-binding oligomerization domain-like receptors. Infectious stimuli include bacterial, viral, and fungal components, while non-infectious stimuli can be components derived from damaged cells or extracellular matrix. A better understanding of the complex sensing and effector mechanisms of innate immune cells in GvHD may help to improve preventive and therapeutic strategies in GvHD.

Introduction
Acute graft-versus-host disease (GvHD) is often preceded by tissue damage due to the conditioning regimen or infectious triggers. However, the disease can also develop in the absence of obvious infection when patients are treated with donor lymphocyte infusions. These observations suggest that the clinical symptoms of GvHD are caused by heterogeneous pathomechanisms that cannot be explained by a single disease model. However, multiple mouse studies and reports on GvHD in patients have indicated that besides the donor T cells as the disease-causing cell type, innate immune cells and the microbial flora participate in GvHD. Damage to barrier tissues such as skin or intestines allows for the translocation of bacterial or fungal antigens and consecutively exogenous and endogenous damage/pathogen-associated molecular patterns (DAMPs/PAMPs) which cause activation of pattern recognition receptors (PRRs) which can lead to the activation of neutrophils [1]. Examples of PRRs include Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) that result in the activation of different types of innate immune cells such as neutrophils, inflammatory monocytes, and macrophages.

Pattern Recognition Receptors in GvHD
The role of different TLRs in GvHD is controversial. Some investigators reported that TLR4 inactivation resulted in reduced dendritic cell (DC) activation leading to reduced GvHD severity [2], and treatment with heparin sulfate an endogenous TLR4 ligand enhanced GvHD [3]. Conversely, others reported that GvHD severity was unaffected by whether host antigen-presenting cells (APCs) were wild-type or deficient in MyD88, TRIF, or MyD88 and TRIF which represent crucial signaling pathways for TLRs [4]. However, the role of TLRs in GvHD is complicated by the fact that also tolerogenic innate immune cells may depend on TLR-based activation, such as for example myeloid-derived suppressor cells which are potent regulators of GvHD [5]. Consistent with a regulatory role of certain TLRs, pretreatment of mice with the TLR5 ligand flagellin resulted in reduced GvHD severity [6]. Administr-
tion of an agonist to TLR-7/8 induced indoleamine 2,3-dioxygenase (IDO) and reduced GvHD-related injury in the colon and ameliorated lethality [7]. Conversely, accelerated GvHD lethality by TLR9 ligation was reported [8]. Consistent with this report, genetic deficiency for TLR9 was associated with reduced GvHD severity [9, 10]. Besides TLRs, cytoplasmic NOD proteins NOD1 and NOD2, the founding members of the intracellular NOD-like receptor family, sense conserved motifs in bacterial peptidoglycan and induce proinflammatory and antimicrobial responses [11]. Penack et al. [12] showed that NOD2 deficiency in host hematopoietic cells exacerbated GvHD. Mechanistically it was shown that proliferation and activation of donor T cells was enhanced in NOD-deficient allogeneic bone marrow transplant recipients and that NOD2 plays a suppressive role in host APCs. In humans, NOD2/CARD15 polymorphisms have been identified as a risk factor for GvHD, although this finding was dependent on the investigated population [13]. PAMPs can be bacterial, viral, and fungal components, and recent evidence suggests a role of fungal antigens for GvHD [14, 15]. Fungal motifs, including β-d-glucan and mannans, can activate the innate immune system via lecin receptors. C-type lectin receptors (CLRs) are defined by the presence of at least 1 C-type lectin-like domain. Of this larger superfamily, the single extracellular C-type lectin-like domain-containing receptors of the ‘dectin-1’ and ‘dectin-2’ clusters associate with signaling adaptors or possess integral intracellular signaling domains and are critical for the recognition of certain fungi [16]. Dectin-1 for example recognizes 1,3-beta-glucans and thereby plays a central role in the sensing of fungal antigens by innate immune cells [16]. The gut microbiota tightly regulates gut barrier function, and recent studies have demonstrated that probiotic bacteria can enhance barrier integrity. In this context, it was shown that Lactobacillus rhamnosus resulted in reduced translocation of enteric bacteria to the mesenteric lymph nodes and was associated with reduced severity of acute GvHD in mice [17]. Conversely, depletion of certain bacteria by treatment with an LPS inhibitor [18] or anti-endotoxin neutralizing antibodies [19] was associated with reduced GvHD severity. Most antibiotics cannot separate beneficial from harmful bacteria. However, prophylactic treatment with fluorochinolone antibiotics during the neutropenic phase for recipients of allogeneic hematopoietic cell transplantation (allo-HCT) is recommended by the European Conference on Infections and Leukaemia with a level of evidence AI [20, 21]. Innate lymphoid cells (ILCs) are likely to be in direct contact with the invading bacteria following allo-HCT. Consistent with a role of ILCs in humans after allo-HCT, it was shown that NCR+ ILC3 cells, which are not present in the circulation of healthy persons, were detectable after induction chemotherapy [22]. This release could be related to intestinal tissue damage which needs to be clarified in future studies.

**Danger Signals and Adenosine Metabolism**

Nucleotides such as adenosine triphosphate (ATP), uridine triphosphate (UTP), adenosine diphosphate (ADP), or uridine diphosphate (UDP) are released by cells that experience stress or damage. The purine nucleoside ATP is highly concentrated in the cell while found in low concentrations in the extracellular space and therefore can serve as a potent danger signal when the cell membrane becomes permeable (reviewed in [23]). The activation of purinergic receptors is tightly regulated by ectonucleotidases, enzymes located on the cell surface which dephosphorylate extracellular nucleotides and eventually metabolize them to the respective nucleosides [24–26]. The ectonucleotidase family that metabolizes the first steps of ATP metabolism, the CD39 family (NTP-Dases), includes enzymes with common motifs in their protein sequences that are able to hydrolyze extracellular ATP and other nucleoside triphosphates (NTPs) as well as nucleoside diphosphates (NDPs) [27]. More downstream, purinergic signaling is modulated by ecto-5'-nucleotidase/CD73, a glycosylphosphatidylinositol-anchored cell membrane enzyme that catalyzes the dephosphorylation of extracellular nucleotide 5'-monophosphates to the respective nucleosides, in particular of 5'-AMP to adenosine [28]. CD73 deficiency of donor or recipient led to significantly aggravated GvHD with reduced survival of the recipient and increased GvHD histopathology score [29] which was later reproduced by another group [30]. Mechanistically, deletion of CD73 caused increased proliferation of alloreactive CD4+ and CD8+ T cells. Consistent with this finding, endogenous adenosine binding to the A2A-AR limited the expansion of alloreactive T cells and dampened the severity of acute GvHD [31, 32]. This was consistent with the finding that activation of the A2A-AR reduced GvHD severity [33]. Increased levels of ATP were found in peritoneal fluids of humans and mice following GvHD or irradiation [34]. Binding of ATP to the purinergic receptor P2X7 leads to potassium efflux with consecutive activation of a multiprotein complex termed Nlrp3 inflammasome. ATP interaction with the purinergic receptor P2X7 on host APCs resulted in increased expression of co-stimulatory molecules CD80/CD86, phosphorylation of signal transducer and activator of transcription (STAT1), and production of inflammatory cytokines [34]. Chimeric mice that were genetically deficient for the P2X7 in host APCs were partially protected from GvHD indicating a critical role for these cells in sensing ATP following allo-HCT [34]. Besides P2X7 receptor-mediated activation, the Nlrp3 inflammasome can be activated by uric acid (UA) [35], a molecule found at high concentrations in patients that develop a tumor lysis syndrome when undergoing chemotherapy. Mice deficient in Nlrp3 or Asc experienced less severe GvHD [36]. Also, depletion of UA reduced IL-1β levels in the serum of mice developing GvHD and GvHD severity when given early after allo-HCT [36]. Consistent with this report in mice, in a recent phase I clinical study patients undergoing allo-HCT received recombinant urate oxidase for 5 consecutive days during conditioning [37]. Patients who developed acute GvHD had a higher level of serum UA in the pretransplantation period compared with those who did not (p < 0.001) [37], and the cumulative incidence of acute GvHD was significantly decreased in the UA depletion group compared to other patients [37]. However, another report found an association of low UA levels with GvHD [38]. Besides the proinflammatory...
roles for certain DAMPs, several lines of evidence have indicated a role for negative regulators of DAMP responses in controlling the severity of GvHD. Sialic acid-binding immunoglobulin-type lectins (Siglecs) are cell surface bound Ig-like lectins that bind sialic acid and function as counter regulators to immune activation [39]. A recent study showed that following total body irradiation for allo-HCT, Siglec-G is expressed on host APCs and can suppress activating signals from DAMPs [40]. Consistent with a negative regulatory role of Siglec-G, mice deficient for this molecule experienced increased GvHD severity. Conversely, enhanced Siglec-G signaling with CD24 in wild-type animals was protective against GvHD [40] indicating that promoting the effects of Siglecs could be exploited to mitigate GvHD severity.

### Therapeutic Targeting of Innate Immune Activation

To manipulate the responses that drive GvHD, a major focus was given to donor-derived T cells. Consequently, the major prophylactic strategies target T cell activation, as for example cyclosporine A [41]. For GvHD treatment, however, the most frequently applied first-line treatment are corticosteroids [42] which target not only T cells but also function and homeostasis of APCs [43]. Therefore, strategies that target innate immune cells may be of added value to conventional drugs used to treat acute GvHD. Spleen tyrosine kinase (Syk) is a non-receptor tyrosine kinase involved in the signal transduction from TLRs, integrins, FcyR, dectin-1, and immunoreceptors such as TCR and BCR. Receptor activation and consecutive phosphorylation of 'immunoreceptor tyrosine activation motifs' (ITAMs) allows for binding and activation of Syk [44]. Syk inhibition did not only impact alloreactive T cell activation but also reduced expression of co-stimulatory molecules on DCs and impaired their migration [45] which was connected to reduced GvHD severity. Recently, a second study confirmed the data and extended it to the chronic GvHD model [46]. Another strategy of targeting APC activation is epigenetic therapy [47]. This approach is based on the assumption that transcription factors that have translocated into the nucleus access anti-inflammatory target genes such as IDO if the epigenetic status of the DNA allows for it. Consistently, histone deacetylase inhibition, which allows for transcription of the respective target gene, was shown to modulate IDO-dependent APC functions and reduce GvHD [47]. These findings in the mouse model were successfully translated into a clinical setting and await further confirmation in a randomized multicenter study [48]. However, epigenetic therapy is not confined to APCs but also impacts regulatory [48, 49] and conventional T cells [50], which could be an advantage given the aggressiveness of severe acute GvHD and the redundancy of different inflammatory pathways. Another approach that is likely to work on multiple levels of the immune response is JAK1/2 inhibition which was shown to reduce GvHD [51]. This approach was motivated by the fact that many of the cytokines involved in acute GvHD such as IL-6 and IL-12 use the JAK/STAT pathway to induce a proinflammatory response. Consistently, myeloid cell activation was reduced by JAK/STAT inhibition by AZD1480 in experimental autoimmune encephalomyelitis [52]. JAK3 inhibition with tofacitinib (CP-690550) [53] or genetic deficiency for JAK3 in donor T cells [54] attenuated GvHD in murine GvHD models. A central event in many pathways of innate immune activation is NF-κB signaling promoting DC maturation and production of immunogenic cytokines (e.g., IL-12, IL-6). Conversely, interfering with NF-κB activation in murine DCs renders them tolerogenic [55]. When APCs are activated, the transcription factors of alternative (RelB/p52) and canonical (c-Rel/p50) NF-κB pathways accumulate in nuclei [56]. Consistent with a functional role of RelB in recipient APCs, bone marrow chimERIC mice lacking RelB in the hematopoietic system developed less severe GvHD [56]. Additionally, APCs from RelB-deficient mice are quantitatively reduced, induce less proliferation, and produce less cytokines compared with wild-type counterparts [56]. P2X7 is expressed by APCs, and lack of the purinergic receptor in recipient type APCs was connected to reduced GvHD severity [34]. Multiple preclinical studies have shown a role of the ATP/P2X7 axis in counteracting inflammation [34, 57–65]. A phase II clinical study on P2X7 inhibition in rheumatoid arthritis showed a significant reduction in swollen and tender joint counts in the P2X7 inhibitor-treated group compared with placebo, whereas no effect on acute-phase response was observed [66]. The less impressive effects as compared to the data from animal
models may be due to the fact that the DAMP ATP is released in higher amounts when severe tissue damage such as in sepsis of GvHD takes place. Another approach for targeting innate immune cells may be via miRNAs (miRs) that control the translation of inflammatory genes. The miRs interact with partially complementary sequences located primarily in the 3'UTR of their target mRNA which can result in either inhibition of translation or degradation of the mRNA which both prevent its translation into the protein/peptide. Multiple inflammation-promoting miRs have been investigated. MiR142–3p was shown to regulate APCs [67] in a model of endotoxin-induced mortality, which can be relevant in GvHD based on the reported role of LPS [18]. Most investigations on miRs in GvHD have focused on donor T cells. MiR142–3p in donor T cells was shown to regulate experimental GvHD via effects on their cell cycle [68]. Also a role of the miR146a/TRAF6 axis in donor T cells for GvHD was shown [69]. Here, genetic deletion of miR146a in donor T cells resulted in repression of TRAF6 with consecutively increased tumor necrosis factor transcription and more severe GvHD [69]. In the clinical setting, we observed that allo-HCT donors, who were homozygous for a miR-146a impairing single nucleotide polymorphism conferred a trend towards a higher risk for severe GvHD grades III and IV in allo-HCT recipients [69]. Also, lack of miR155 in donor T cells was connected to reduced levels of GvHD, and miR155 was found in the intestinal tract of patients developing GvHD [70]. However, not only T cells were investigated with respect to a proinflammatory role of miRs in GvHD. We recently showed that miR-100 can regulate inflammatory neovascularization during GvHD [71] suggesting its role in endothelial cells. To exploit the pro- or anti-inflammatory functions of different miRs in acute GvHD, clinical-grade miR mimetics or antagonists need to be developed. The multiple targets for GvHD treatment based on preclinical and early clinical trials are summarized in figure 1.

Disclosure Statement

The author has no conflict of interest to disclose.

References
