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Acute Graft-versus-Host Disease Biology, Prevention and Therapy

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Acute GVHD - clinical presentation and risk factors

Allogeneic hematopoietic cell transplantation (allo-HCT) is a well-established treatment for hematological diseases incurable by conventional treatments[1]. More than one million hematopoietic cell transplants have been performed, of which 40% were allogeneic[2]. The most common life-threatening complication is graft-versus-host disease (GVHD). GVHD occurs when immune competent T cells in the donated tissue (the graft) recognize the recipient (the host) as foreign (nonself). The resulting immune response activates donor T cells to gain cytolytic capacity then attack the recipient to eliminate foreign antigen(s)-bearing cells. The two main clinical presentations are acute GVHD and chronic GVHD. Typical acute GVHD signs include a maculopapular rash (skin), hyperbilirubinemia with jaundice due to small bile duct damage leading to cholestasis (liver), nausea, vomiting and anorexia [upper gastrointestinal tract (GI)], and watery or bloody diarrhea and crampy abdominal pain (lower GI) (Suppl. text, Suppl. Table 1 in Suppl. Appendix; Figure 1). Acute GVHD diagnosis relies on clinical, laboratory, and biopsy assessment of target organs. Acute GVHD severity is graded clinically by tabulating the extent of involvement of the three main target organs: skin (the most frequent and often the earliest clinical manifestation of acute GVHD), gastrointestinal tract (second most common), and liver[3,4]. Overall grades are grade I (mild), II (moderate), III (severe), and IV (very severe). Amongst all allogeneic hematopoietic cell transplant patients, 30-50% develop acute GVHD (grade I-IV) and 14% experience severe acute GVHD (grade III-IV)[5]. Risk factors are summarized in Table 2 (Suppl. Appendix) and include degree of HLA mismatch, unrelated donors, female donors for male recipients, peripheral blood stem cell grafts and conditioning regimen intensity[6–8].

Donor T-cell recognition that induces acute GVHD can be directed against host MHC and/or minor histocompatibility antigen (miH) disparities. HLA class I molecules (A, B, and C) are expressed at variable levels by all cells whereas MHC class II molecules (DR, DQ, and DP) are mainly expressed by hematopoietic cells, especially antigen-presenting cells (APCs)

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such as host B-cells, dendritic cells (DC), macrophages and monocytes. Recent data indicate that recognition of a mismatch in the polymorphic MHC class I chain-related gene A or MICA is connected to higher GVHD risk[9]. The role for miH is supported by genome-wide analysis of single nucleotide polymorphisms (SNPs) resulting in amino acid coding differences between recipients and donors[10]; each 1% increase in genome-wide recipient mismatching is associated with a 20% increase in the hazard of severe acute GVHD[10]. MiH mismatches and allo-hematopoietic cell transplant are connected to GVHD[11]. Although high-resolution HLA typing with next generation sequencing will likely detect more HLA gene mismatches, it is unclear whether such information will lead to improved allo-hematopoietic cell transplant outcomes, since not all mismatches may be recognized by the donor T cells. Additionally, SNPs for chemokines, cytokines, costimulatory molecules and micro-RNAs (miRs) are also associated with acute GVHD risk (Suppl text). Although acute GVHD risk can be increased by mismatches and closer matching may reduce the risk, mismatched antigens present in normal tissue may be shared with malignant cells; thus, some mismatches may be important for graft-versus-leukemia (GVL) responses and greater matching could increase relapse risk.

Tissue damage and early events of acute GVHD investigated in the mouse model

Our understanding of acute GVHD biology is mostly based on murine studies, albeit limited by the physiological and immune system differences of mice and humans, transplant procedures[12], and microbiome[13]. Nonetheless, GVHD mouse models have been the basis for much of our understanding of GVHD biology. The earliest acute GVHD pathophysiological events are neoangiogenesis[14,15] and intestinal tract infiltration by innate myeloid cells such as neutrophil granulocytes (neutrophils)[16–19] and monocytes[20,21], first-wave immune responders to tissue injury and foreign pathogens. Recipient neutrophils impact GVHD through their activation and reactive oxygen species (ROS) production in the GI tract[17]. The impact of neutrophils on acute GVHD is further supported by observations that a high density of neutrophil infiltration correlated with an unfavorable outcomes[18], increased intestinal permeability after allo-hematopoietic cell transplant[19] and conversely, defective neutrophil ROS production in chronic granulomatous disease patients resulted in low acute GVHD rates[22]. However, tissue protective effects can be conferred by neutrophils and monocytes dependent upon the specific cell subset involved, time point related to allo-hematopoietic cell transplant and the tissue and environment context (Suppl. text, Suppl. Appendix).

In the early acute GVHD phase, inflammatory triggers can drive both the innate and adaptive immune responses. These triggers can be divided into sterile damage associated molecular patterns (DAMPS) and pathogen-associated molecular patterns (PAMPS). The DAMPS comprise molecules released into the extracellular space only when tissue damage occurs that cause immune activation[23]. GVHD can be enhanced by extracellular adenosine triphosphate (ATP) activating the purinergic P2X7 receptor[24] and P2Y2 receptor[20]. ATP is metabolized by the ectonucleotidase CD39, expressed by endothelial and immune cells, into adenosine monophosphate and then into anti-inflammatory adenosine (by CD73, expressed

in acute GVHD organs such as colon, liver, and lung; endothelial cells; leukocytes)[25]. Consequently, the lack of CD73 enhances GVHD but also GVL effects[26,27]. ATP and uric acid can cause activation of the Nlrp3 inflammasome, a myeloid expressed multiprotein oligomer containing caspase-1 or -11, leading to pro-IL-1β cleavage into its bioactive form, enhancing GVHD[28]. Other DAMPs include heparan sulfate, high mobility group box 1 protein (HMGB1), sialic acid-binding immunoglobulin-type lectins (siglecs), mitochondrial components, IL-33 or the small leucine-rich repeat proteoglycan, biglycan[29–36] (Figure 2). Like myeloid cells, tissue and inflammatory context are important for the net DAMP effects. This was particularly evident for IL-33 that has anti-inflammatory properties when given before tissue damage due to expansion of IL-33 receptor [suppressor of tumorigenicity (ST2)] expressing suppressor $CD4^+CD25^+FoxP3^+$ regulatory T cells (Treg)[34]. Conversely, IL-33 administration during evolving GVHD promotes interferon (IFN)-γ producing T cell expansion and acute GVHD[34], while blockade of the IL-33/ST2-axis can reduce acute GVHD[34,35].

Although the role of sterile DAMPS in GVHD was discovered in the last decade, the role of bacteria and the resulting PAMPS such as lipopolysaccharide (LPS) in GVHD has been studied since the 1970s with the first seminal studies in mice[37]. Direct effects of bacterial components that engage immune system pattern recognition receptors (PRRs) such as Toll-like receptors (TLR) and nucleotide-binding oligomerization domain-like receptors (NOD-like receptors; NLRs) can activate APCs, promoting acute GVHD. Novel technologies such as bacterial genome sequencing permit better understanding of which bacteria in the GI tract decline with antibiotics diminishing acute GVHD and conversely, which bacteria have a protective role for acute GVHD[38,39] as well as relapse- and progression-free survival[40]. GVHD itself induces dysbiosis in mice[41]. Increased GVHD-related mortality that can occur in mice and in patients due to antibiotic treatment[39] is likely due to the loss of GI homeostasis that heavily depends on microbiota-derived metabolites. Intestinal bacteria-secreted butyrate functions as a histone deacetylase (HDAC) inhibitor, and potently reduces GVHD by inhibiting indoleamine-2,3-dioxygenase (IDO)-dependent innate immune and allo-stimulating APC functions in a STAT-3-dependent manner[42]. IDO expession itself is upregulated on intestinal parenchymal cells and APCs in mice[43] as a result of IFN-γ produced by alloreactive T- cells[44]. IDO causes local depletion of the essential amino acid tryptophan through a stress response mechanism, resulting in T-cell metabolic starvation and apoptosis at sites of high IDO expression (e.g. colon)[43]. Fungi and viruses also are connected to GVHD severity, e.g. α-Mannan derived from fungi induces Th17-mediated pulmonary GVHD in mice[45]. Cumulatively, DAMPS and PAMPS lead to rapid intracellular biochemical cascades that induce caspase-1 cleavage of inactive cytokines stored intracellularly (e.g. IL-1β; IL-18) and the transcription of genes that encode for cytokines, chemokines and their receptors. Overall, the early events of acute GVHD set the stage for later T-cell priming and expansion.

T-cell activation, co-stimulation, survival and metabolism in mouse models of aGVHD

A key event for the development of acute GVHD is the interaction of T cells expressing a suitable T-cell receptor (TCR) with APCs that express host MHC or miH peptides. Recent reports from mouse acute GVHD models also point to the role of non-hematopoietic cells in the antigen presentation process[46,47]. T cells are the main effectors causing target tissue cell death that can be mediated by the expression of the TNF family member, FAS-ligand, and release of intracellular granule contents including the serine protease, granzyme B, and pore-forming cytolytic protein, perforin[48,49]. Where MHC class I only is mismatched, donor $CD8^+$ T cells alone are sufficient to induce GVHD. For miH disparities, $CD8^+$ T cells require cognate (direct) interactions with GVHD target tissues miH[50], a situation particularly relevant in human allo-hematopoietic cell transplant recipients due to frequent miH mismatches. $CD4^+$ T cells can cause GVHD by cognate interactions with MHC class II alone or with miH peptides or can damage tissues without cognate interactions by releasing cytotoxic cytokines, such as TNF-α, that induce apoptosis in epithelial cells[51]. In contrast to T cells, NK cells reduce GVHD via the elimination of recipient-type APC based on killer-cell immunoglobulin-like receptor (KIR)-mismatches[52] and elaboration of TGFβ that can suppress T-cell activation[53].

Besides the TCR activation, T cells need co-stimulation, a second T-cell signal required to lower the TCR activation threshold, amplify and sustain cytokine production, inhibit apoptosis, and support T-effector metabolism. The role of multiple costimulatory pathways has been studied in acute GVHD including positive regulatory axes [CD28[54,55], ICOS (CD278)[56]], TNFR-superfamily receptors [CD40L(CD154), OX40(CD134), 4-1BB(CD137)] and negative regulatory pathways [CTLA-4(CD152)[55], PD-1(CD279)/PD-L1(CD274)[57,58] and B7-H3[59](CD276)], amongst others (Suppl. text, Suppl. Appendix). The third signal for the T-cell activation and survival is cytokine-mediated. Multiple cytokines (Suppl. text, Suppl. Appendix) were found to play a role in the pathogenesis of GVHD such as IL-1β and T-helper 1 (Th1) cytokines (IFN-γ, IL-2, and TNF). The differentiation stage (naive, effector/memory) of T cells is decisive for their ability to cause acute GVHD. In acute GVHD mouse models, memory $CD4^+$ T cells cause less or almost no acute GVHD but mediate GVL effects[60,61]. Naïve T cells migrate to lymph nodes, via L-selectin (CD62L) and the chemokine CCR7, where priming takes place, e.g. via interaction with donor-derived $CD11b^-CD103^+$ DCs that have migrated to mesenteric lymph nodes from the colon, imprinting donor T cells to express gut-homing integrin receptors[62].

T-cell differentiation and proliferation during GVHD require multiple energy sources to keep pace with the high metabolic demands. Aerobic glycolysis is essential for optimal GVHD T_{eff} responses[63–65]. In some studies, T_{effs} also have been shown to utilize oxidative phosphorylation and fatty acid oxidation[63,65,66], supplying vital energy needs not accomplished by glycolysis alone. Targeting metabolic pathways and subverting T-cell energy utilization by inhibition of glycolysis, fatty acid oxidation and oxidative phosphorylation, and/or glutaminolysis or via essential or conditionally essential amino acid deprivation (e.g. tryptophan or L-arginine, respectively) may reduce the frequency of rapidly

proliferating T cells responsible for acute GVHD. This field of investigation builds upon early studies demonstrating that blockade of the nutrient sensor, mammalian target of rapamycin (mTOR), ameliorates acute GVHD[67] and CD28 costimulation regulates glycolysis[68] along with more recent studies showing that the negative regulator, PD-1, suppresses glycolysis and promotes lipolysis and fatty acid oxidation[69]. The specific nutrients and magnitude of energy needed during GVHD likely depends upon the T-cell subset, proliferation rate, intensity of inflammatory response, and local tissue environment. As metabolism drives cell cycle and proliferation, other novel targets are cyclin-dependent kinases (CDK) that affect cell cycle regulation. For instance the CDK-inhibitor roscovitine prevents alloreactive T-cell expansion and protects against acute GVHD in mice[70]. The role of the three signals for T-cell activation and differentiation, key cytokines, and metabolic demands derived from murine acute GVHD studies provide pathophysiology-based targets for exploration in humans.

Biomarkers for aGVHD severity - studies in patients

To predict the risk of GVHD and the response to immunosuppressive therapy, multiple biomarkers have been investigated in allo-hematopoietic cell transplant patients (Suppl. text, Table 3, Suppl. Appendix). The serum level of the soluble form of ST2 was reported to be an important biomarker for therapy-resistance in patients developing acute GVHD[71]. Based on this observation an early-biomarker algorithm was studied for its predictive value for lethal acute GVHD[72]. A 4-biomarker panel [ST2, TNFR1, IL-2Rα chain(CD25), regenerating islet-derived protein-3 alpha(REG3α)], released from injured tissue or activated T_{effs}, was used to predict increased acute GVHD-related death. By modeling 6-month non-relapse mortality in an independent test set and validation set, a 2-biomarker model using ST2 and REG3α concentrations identified patients with a cumulative incidence of 6-month nonrelapse mortality of 28% in the high-risk and 7% in the low-risk group[72].

Micro-RNAs (miRs) are potent regulators of multiple pro-inflammatory target genes and are readily measurable in patient serum. Multiple miRs in sera were strongly connected to acute GVHD risk[73], in particular miR-155 and miR146a[74]. In initial studies, the presence of a miR-146a polymorphism (rs2910164) in the donor or the allo- hematopoietic cell transplant recipient was connected to higher rates of grade III and IV acute GVHD[75,76], a finding requiring confirmation in larger patient cohorts. These and possibly other yet to be discovered biomarker panels hold promise to better predict the risk of acute GVHD and acute GVHD-related mortality, which could lead to a more individualized GVHD-prophylaxis approach.

Classical acute GVHD preventive and therapeutic strategies that have been tested in the clinic

Acute GVHD prophylaxis with the calcineurin inhibitor cyclosporin A and methotrexate, a folate antagonist, is used in the majority of allo-hematopoietic cell transplant recipients based on a sequential, prospective randomized trial showing cyclosporine A and methotrexate was superior to cyclosporine A alone[77] (Suppl. text, Suppl. Appendix). Randomized, multicenter phase-III trials of the pan-T-cell-depleting reagent, rabbit anti-

thymocyte globulin (ATG-F), showed decreased acute GVHD and chronic GVHD incidence without increased relapse or non-relapse mortality when added to standard prophylaxis[78,79]. The mode of action of the most frequently applied classical immunosuppressive medications is summarized in Figure 3A. The cyclosporine A and FK506, inhibit GVHD by preventing nuclear factor of activated T cells (NFAT) activation thereby reducing IL-2 transcription and T_{eff} activation, albeit with a concurrent reduction in anti-inflammatory Treg that are IL-2-dependent. In contrast to calcineurin inhibitors, rapamycin is more potent in suppressing expansion of conventional T cells compared to Treg, most likely due to their greater dependence on the mTOR/protein kinase-B-pathway compared to Tregs[80]. More recently, post-transplant cyclophosphamide has been used to deplete conventional T cells while relatively preserving Tregs[81], resulting in a relatively low incidence of GVHD even in haploidentical transplant recipients[82].

Cell-based approaches to prevent acute GVHD include the manipulation of the donor graft. Ex vivo T-cell depletion accomplished by positive selection of $CD34^+$ cells or negative selection against T cells and B cells was reported[83]. A promising strategy is the enrichment of T-cell receptor gamma/delta T cells to prevent relapse[84]. T-cell-depletion specifically for naive T cells alone showed a reduced chronic GVHD incidence[85]. Adoptive transfer of Treg or donor lymphocytes cultured with IL-10-treated host APCs that enriches for IL-10 and TGFβ producing Tregulatory type 1 (Tr1) has been investigated. Clinically, prophylactic Treg transfer was associated with low acute GVHD rates and adequate immune reconstitution[86,87]. Tr1 enhanced immune reconstitution in 5/12 patients when given at 1-2 months after allo-hematopoietic cell transplant[88]. Besides Treg and Tr1, mesenchymal stroma cells were tested in the clinic for their ability to reduce GVHD severity. Different clinical studies showed either impressive responses or, in a randomized clinical trial, failed to show improvement of GVHD-related mortality[89,90] which may be due to mesenchymal stem cell preparation, transfer time point, GVHD severity or organ involvement.

Despite prophylaxis acute GVHD still evolves and is treated first with glucocorticoids based on randomized controlled trials[91]. Acute GVHD patients that are glucocorticoid refractory have a dismal long-term prognosis with only 5-30% overall survival. A summary of acute GVHD drug therapies is listed in the supplementary appendix. Currently only two randomized phase-III trials in glucocorticoid-refractory acute GVHD have been reported using gavilimomab (murine anti-CD147; ABX-CBL)[92] or inolimomab (murine anti-CD25)[93]. The first trial reported an 18-month overall survival after treatment initiation that was less favorable in the gavilimomab arm compared to the ATG arm (35% versus 45%)[92]. In the second trial, inolimomab was compared to ATG with the primary objective to evaluate 1-year overall survival[93]. The primary end point was not reached and there was no significant difference in overall survival[93]. As both antibodies had been promising in early phase clinical studies but failed to show improvement in phase-III trials, it is important that promising agents used to treat glucocorticoid-resistant acute GVHD identified in phase I/II or phase II trials are tested in randomized phase-III trials in the future. As such, no proven second-line therapy has been uniformly adopted or approved for glucocorticoid-resistant acute GVHD.

Novel acute GVHD preventive and therapeutic strategies being tested

Since T-cell migration to GVHD organs is needed to cause acute GVHD and is initiated by chemokine gradients produced within these target organs as a result of tissue injury or innate cell infiltration, chemotaxis should be considered a central event in acute GVHD pathogenesis. In support of this contention, a clinical phase-II trial reported that CCR5 inhibition prevents GVHD of liver and gut before day 100[94]. The phase-II trial used reduced-intensity conditioning which may be relevant to the successful outcome because the CCR5 migratory signals appear less important in the context of myeloablative radiation in mouse acute GVHD models. For example, CCR5 inhibition was protective against GVHD in a non-irradiated GVHD mouse model but GVHD onset was earlier and severity worsened when CCR5-deficient T cells were transferred into heavily irradiated GVHD model[95]. In a different approach to inhibit migration, the sphingosphine-1-phosphate receptor antagonist, FTY720, has been shown to reduce murine acute GVHD by trapping T cells in lymphoid organs or reducing DC migration[96] and is currently in clinical trials to prevent acute GVHD.

The fundamental role of IL-22 has been explored. IL-22 is produced by innate lymphoid cells type-3 (ILC3) that are depleted by GVHD, resulting in cypt apoptosis, ILC3 depletion and epithelial integrity loss[97–99]. Exogenous IL-22 can enhance the regeneration of intestinal stem cells (ISC) that express IL-22 receptors[97–99]. In a clinical trial (NCT02406651,ClinicalTrials.gov), IL-22 IgG2-Fc(F-652) is being given to patients with grade II-IV acute GVHD of the lower intestinal tract. Another recent approach to protect the intestinal tract involves the direct transfer of microbial species as acute GVHD therapy. In a small pioneer study, the first successful and safe application of related fecal microbiota transplants via nasoduodenal tubes in patients suffering from glucocorticoid-resistant acute GVHD was reported[100]. Three of four patients responded by 28 days after the first fecal transplant, allowing reduction of the glucocorticoid dose by 69%[100]. While encouraging, fecal transplantation is still to be considered a highly experimental treatment approach and needs validation in carefully designed prospective clinical trials. Other approaches currently being explored to prevent or treat GVHD in patients are blockade of T-cell co-stimulation[101], α-GalCer, a glycolipid that expands and activates natural killer T cells and subsequently expands Treg in patients[102], anti-inflammatory antibodies, proteins or drugs targeting signaling by IL-6[103], IL-23[104], or multiple cytokine signaling pathways using HDAC inhibitors[105], proteosomal inhibition[106], or the anti-inflammatory protease inhibitor, alpha-1-antitrypsin[107]. Additionally, Janus-activated kinase (JAK)-1/2 inhibitors have shown promising results in preclinical studies[108,109] as well as in retrospective clinical analyses[110] and are currently being tested in prospective randomized studies.

Promising novel strategies against acute GVHD tested in preclinical models but not yet in GVHD trials

In contrast to the approaches that have already reached clinical application, preclinical strategies being tested have continued to focus on targeting the signalling of multiple cytokine receptors, pro-inflammatory pathways and intestinal stem cells. Novel targets include kinase inhibitors that block the protein serine/threonine kinase ROCK1[111], Aurora

kinase A[112], MEK[113], and others (Supplemental appendix and Figure 3B). Strategies being investigated to improve Treg efficacy by in vivo Treg expansion in mouse models include TNFRSF25 (DR3) stimulation using a fusion protein to ligate the receptor[114], agonistic DR3 antibody[115] and agonistic TNFR2 antibody[116].

Administration of R-spondin-1(R-Spo1), a WNT agonist, to mice reduced GVHD by protecting intestinal stem cells from conditioning injury[117] and stimulating them to differentiate into secretory cells thereby inhibiting GVHD-associated microbiome changes. The protective effect of R-Spo1 was connected to the microbial microflora in the intestinal tract and partly abrogated when mice received broad-spectrum antibiotics. While bacterial components can activate innate immune cells like neutrophils and monocytes, bacteria are also critical for intestinal tissue homeostasis. Indeed, transfer of selected strains of *Clostridia* known to produce the short chain fatty acid butyrate results in increased Treg frequencies in the GI tract[118]. Butyrate given via the GI tract may be proven to be effective in the absence of *Clostridia* transfer, albeit repetitive administration would likely be needed during the period of acute GVHD risk. Since Treg can potently suppress acute GVHD,[119,120] these data link intestinal metabolism to GVHD. Transfer of innate lymphoid cells type 2 (ILC-2) into mice both prevented and treated acute GVHD by stimulating the expansion of anti-inflammatory regulatory cell populations[121]. While still in pre-clinical testing, these approaches may be more effective than classical broadly immunosuppressive strategies compared to targeting of individual cytokine or chemokine signals or preferentially targeting the GI tract.

Although pharmacological strategies to overcome acute GVHD inflammation are typically short-lived unless a state of deep tolerance is acquired during drug therapy, the transfer of a tolerogenic cell population that persists in the body, could ideally lead to the achievement of long-term tolerance. First steps towards this strategy were made when Treg adoptively transferred into mice reduced acute GVHD[119,120].

Summary and outlook

Acute GVHD remains a major life-threatening allo-hematopoietic cell transplant complication leading to high mortality and rendering patients that survive often profoundly immune deficient for several years. Acute GVHD clinical diagnosis, pathophysiology, standard as well as experimental prevention and treatment procedures and novel biomarkers to tailor GVHD-treatment are important developments that hold promise to lead to reduced acute GVHD rates. Major pathophysiologic pathways that drive acute GVHD include tissue damage due to the conditioning regimen or infection, recognition of non-self-MHC/miH and altered repair/tissue protective mechanisms including microbiome changes that cause a decline in protective microbial-derived metabolites.

Therapeutic directions that are particularly promising to pursue based on early clinical trial data include costimulatory pathway blockade, anti-IL-6R mAb[103], HDAC-inhibitors[105], kinase[108,109]- and proteasome-inhibitors[106], the anti-inflammatory protease-inhibitor alpha-1-antitrypsin[107], CTLA-4 antagonism[101], CCR5 blockade[94] and adoptive Treg

transfer[86,87]. These and other novel strategies being developed have to be tested in prospective phase-III trials to become standard therapy for acute GVHD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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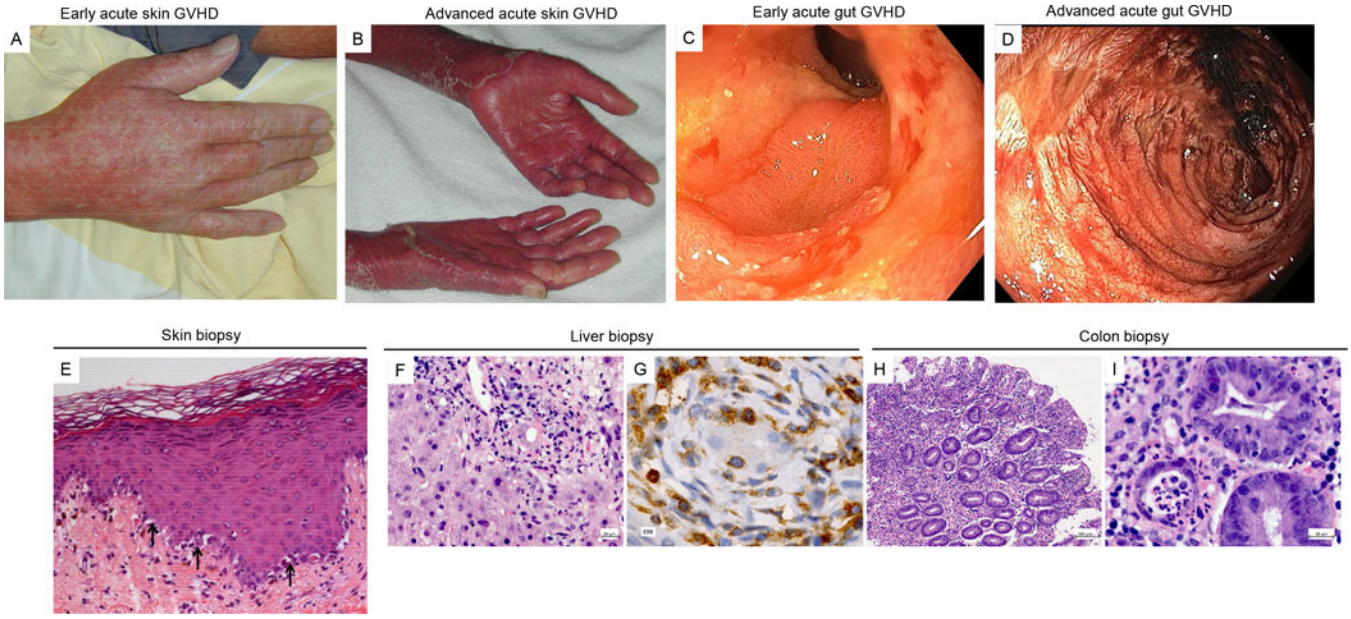

Figure 1. Clinical features of aGVHD

Representative pictures for clinical aGVHD in early and advanced stages of the skin and intestinal tract are shown (A-D). Histologic aspects of skin (E), hepatic (F, G) and intestinal (H, I) lesions in GVHD. E. AGVHD of the skin, the black arrows indicate apoptotic cells in the basal layer of the epidermis. F. Damage to small interlobular bile ducts characterized by epithelial irregularities and rare apoptosis. Moderate inflammation of the adjacent portal area (hematoxylin & eosin, medium magnification). G. Infiltration of bile duct epithelium by $CD3^+$ lymphocytes (anti-CD3 immunolabeling, high magnification). H. Mucosal surface denudation and partial crypt destruction in intestinal GVHD (hematoxylin & eosin, low magnification). I. Scattered apoptotic bodies in regenerating crypts in close association with exploding crypts containing karyorrhectic nuclear debris (hematoxylin & eosin, high magnification). Histological images were provided by Dr. Technau, Dept of Dermatology, Univ of Freiburg (skin), and Prof Schmitt-Gräff, Dept of Pathology, Univ. of Freiburg (liver, intestines).

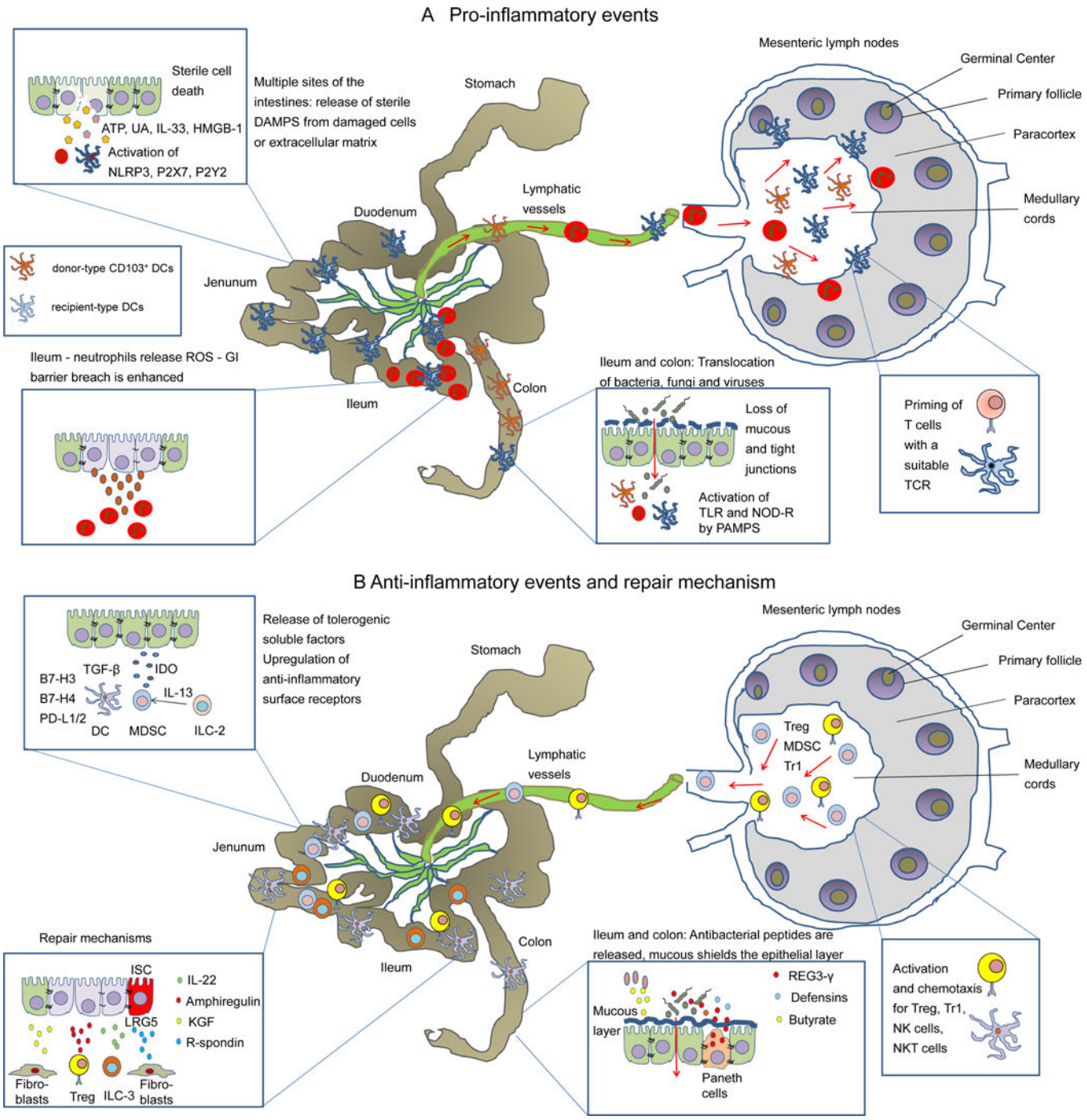

Figure 2. Schematic overview of the early events of GVHD

A: DAMPs (e.g. uric acid, ATP, heparan sulfate, HMGB-1 or IL-33) that are released from the dying cells or disrupted extracellular matrix and activate the respective receptors, e.g. ATP activates P2X7 and P2Y2, uric acid activates the Nlrp3 inflammasome. PAMPS derived from invading bacteria activate innate immune cells including donor derived $CD103^{+}$ dendritic cells, inflammatory monocytes and neutrophils. A fraction of these cells migrates from the damaged intestinal epithelium towards the draining mesenteric lymph nodes where

donor T-cells are activated. ATP: Adenosine triphosphate, HMGB-1: High mobility group box 1 protein, ROS: reactive oxygen species, DAMPs: danger associated molecular patterns, PAMPs: pathogen associated molecular patterns.

B: Anti-inflammatory events and repair mechanism. Cells in the GVHD target organs attempt to counterbalance inflammation via the release of tolerogenic soluble factors, upregulation of anti-inflammatory surface receptors and repair mechanisms. Activation signals and chemotactic signals for Treg and Tr1 cells are provided in the lymph nodes. KGF: keratinocyte growth factor.

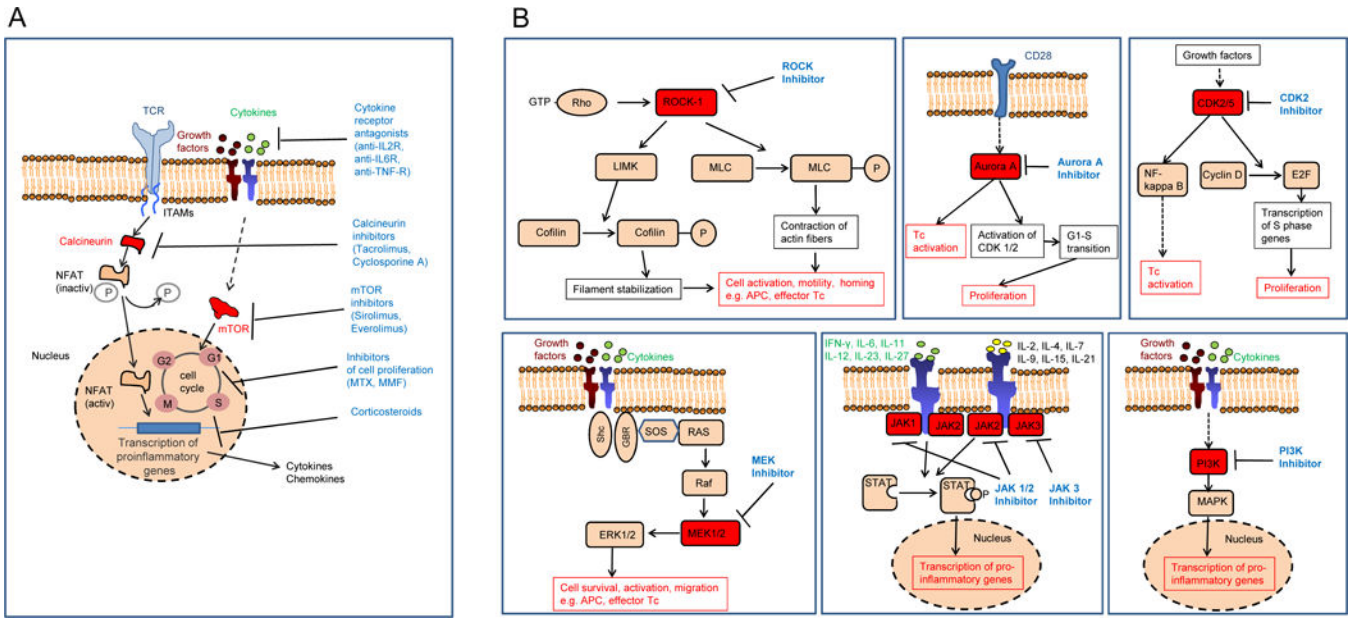

Figure 3. Classical and novel approaches to target T-cell and dendritic cell activation

A: Sketch showing the mode of action of multiple immunosuppressive strategies that are currently applied in the clinic for prevention and therapy of aGVHD. mTOR: mammalian target of rapamycin, MTX: methotrexate, MMF: mycophenolate mofetil

B: Kinases that have been subject to targeted therapy approaches in aGVHD are shown. Blockade of the kinases ROCK-1, Aurora A, CDK2, MEK-1/2, JAK1-3 and PI3K was shown to reduce aGVHD in mouse models. The different signaling pathways in which these kinases have a non-redundant function are displayed. Tc: T cell, TCR: T-cell receptor